Impacts of habitat and landscape characteristics on reproductive ecology of female lesser scaup (*Aythya affinis*) in the boreal forests of Alberta

A Thesis Presented to the

College of Graduate Studies and Research at the

University of Saskatchewan

In partial fulfillment of the requirements for the Master of Science Degree

In the Department of Veterinary Biomedical Sciences

Western College of Veterinary Medicine

By

June Z. Yang

PERMISSION TO USE

In presenting this thesis/dissertation in partial fulfillment of the requirements for a Master's degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis/dissertation in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis/dissertation work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis/dissertation or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis/dissertation.

DISCLAIMER
Reference in this thesis/dissertation to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement, recommendation, or favoring by the University of Saskatchewan. The views and opinions of the author expressed herein do not state or reflect those of the University of Saskatchewan, and shall not be used for advertising or product endorsement purposes.
Requests for permission to copy or to make other uses of materials in this thesis/dissertation in whole or part should be addressed to:

Head of the Department of Veterinary Biomedical Sciences
Western College of Veterinary Medicine
52 Campus Drive
University of Saskatchewan
Saskatoon, Saskatchewan, S7N 5B4
Canada

OR

Dean
College of Graduate Studies and Research
University of Saskatchewan
107 Administration Place
Saskatoon, Saskatchewan S7N 5A2
Canada

ABSTRACT

The lesser scaup (*Aythya affinis*, henceforth scaup) population decreased during the 1980s and has remained below conservation objectives. With two-thirds of the breeding population nesting in boreal forests, it is imperative to understand how changes in habitat features and landscape changes could negatively impact breeding scaup; this information could also help to guide waterfowl conservation and management programs. It has been hypothesized that landscape modifications, such as agricultural and oil exploitation activities, could adversely affect scaup reproductive performance. Female scaup were collected by shooting in the boreal forest fringe of central Alberta during within the first two weeks of June 2008 and 2009, corresponding to the early laying period. Collection location was determined using a hand-held GPS device and body mass (BM, g) was recorded immediately. When scaup carcasses were dissected, ovaries were removed and weighed. Rapidly growing follicles (RGF) and oviductal follicles (OF) were subjected to radioimmunoassay to quantify amount of ovarian corticosterone (OCORT) deposited into follicles. Nest initiation date (NID) was determined by counting and subtracting the number of postovulatory follicles (POF) from collection date.

Natural landscape features and habitat disturbances within 100 m, 250 m, 500 m, 1500 m, and 5000 m buffers of each scaup collection location were quantified, and then normalized using z-score transformation. General linear modeling was applied to BM, OCORT and NID across each of the five buffer sizes using *a priori* candidate models. Natural landscape and habitat disturbance parameters were model-averaged to obtain 85% confidence intervals to determine which habitat features best predicted BM, NID, and OCORT. Model-averaging revealed that the amount of natural water body habitats were negatively correlated to scaup OCORT at the 100 m

and 500 m buffer zones, and negatively correlated to scaup BM at 1500 m and 5000 m buffer zones. A combination of natural water bodies (BOGSFENS, SWAMP, WATER, and WETLAND) were negatively correlated to NID across all buffer sizes tested, AGRIC disturbance was positively correlated to NID at the 100 m and 1500 m buffer zones, WELLS were negatively correlated to NID at the 1500 m buffer zone, while TRANSPORT showed varied responses at the 1500 m and 5000 m buffer zones. Post-hoc exploratory analyses were conducted to assess whether variation in OCORT, BM, and NID was related to habitat features operating at more than one spatial scale. This analysis revealed models incorporating more than one spatial scale were competitive when compared to the original *a priori* model sets at the 250 m buffer zone, with post-hoc models performing better than a priori models. This study highlights the need for a holistic approach to conservation management, considering not only local habitat characteristics and disturbances in the immediate vicinity of breeding waterfowl, but extending beyond and incorporating regional landscape attributes.

ACKNOWLEDGEMENTS

As with the many other endeavors I have undertaken, I could not have completed my thesis without the assistance from some very talented and amazing individuals. I would thus like to take this opportunity to thank my supervisor Dr. Karen Machin for her endless support, encouragement, and guidance throughout the last four years. Dr. Machin has truly been an inspiration for me, and I hope to contribute positively to the world of wildlife conservation following her example. I would also like to thank the members of my graduate committee Drs. Gillian Muir, Marc Cattet, and Robert Clark for their continued support, feedback, and guidance throughout my Master's degree. Their thesis was made possible by their knowledge, experiences and contributions to which, I am eternally thankful. I am forever indebted to Mark Bidwell, Robert Brua, and Robert Clark for their patience and bestowing their endless knowledge of statistical analysis, methods, and landscape ecology on me. I would like to thank both Susan Cook and Kim Tran of the Veterinary Biomedical Sciences Endocrine Laboratory for their assistance in the development of the project protocols and laboratory procedures. To Keith Brown and Gabrielle Schatte of the Saskatchewan Structural Sciences Centre, thank-you for your time and assistance in helping me become familiar with the Nuclear Magnetic Resonance Spectroscopy program and equipment. A special thank-you to Dr. Erin Bayne from the University of Alberta for providing habitat data specific to our areas of scaup collection, and thank-you to Dr. Adam G. Yates from Western University for extracting vital habitat information and attributes from these layers. To my numerous field assistants Robert Brua, Kerry Edwards, Steve Leach, Raghavun Premkumar, Alana Redfern, and Shane Roesma; thank you for all your hard work in the field, and for waking up so early in the mornings. Special thanks to Erin

Langwith, who conducted field collections in my absence in the field season of 2008. Special thanks to my friends and supporters in Ontario who are too numerous to list, and to my friends in Saskatoon Michelle Scott, Nikki Stephenson, and Gillian Gratton who made these past few years truly memorable.

Last but not least, I would not have been able to complete my thesis without the constant support of my fiancé Blake MacGowan, whose countless words of encouragement have and always will be remembered. And most of all to my wonderful loving (albeit sometimes dysfunctional) family, for their constant support and for never failing to put a smile on my face; my deepest thanks to my parents and my brother Kevin and sister Amy. This thesis is dedicated to you, my family, for always believing in me, and for the countless journeys and misadventures we will continue to pursue together.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 LITERATURE REVIEW

1.1 Introduction:

Of the many challenges facing wildlife conservation and management, habitat loss and degradation remain one of the primary threats to wild avian species (Afton, 1984; Johnson, 2007). Methods of assessing habitat change impacts on avian species have included quantification of physical habitat attributes (Brandt, Parrish, & Hodges, 1995; Whittingham, et al., 2005) and studies assessing changes to community relationships/structure (Fagan, Cantrell, & Cosner, 1999; Hockin et al., 1992) and/or life history (Hockin et al., 1992). Understanding variation in individual physiological responses and their subsequent effects on important life history stages (such as reproduction) is vital in order to more fully understand how these habitat changes affect entire wildlife populations.

Modifications to the landscape or repeated disturbances arising from human development can alter survival and reproduction in birds. In response to alterations of the environment, birds may release the stress hormone, corticosterone (CORT), which alters behaviour and physiology to allow the individual to cope with these changes (viewed as stressors). Measuring circulating levels of CORT may be used to measure habitat quality attributes. For example, American redstarts (*Setophaga ruticilla*) segregated disproportionately into different habitat types (predominately males found in black mangrove habitat, and females in logwood habitats) demonstrated variability in CORT responses by location of collection (Marra & Holberton, 1998). Redstarts (of all ages/sexes) sampled in the logwood-habitat during the spring demonstrated higher baseline levels of CORT compared to redstarts found in the black mangrove

habitats. In addition, redstarts in the logwood habitat demonstrated a lower levels of CORT in response to handling/restraint stress compared to redstarts in the black mangrove habitat; possibly indicating a desensitization of the CORT response (Marra & Holberton, 1998). CORT has been used in other species as a measure of the impact of habitat disturbance. Spotted salamanders (*Ambystoma maculatum*) migrating to breeding ponds over pavement had higher baseline CORT responses compared to salamanders migrating through a forest (Newcomb-Homan et al., 2003). This result was viewed as a direct response to the habitat alteration and may indicate that changes to habitat are acting to stress certain wildlife species.

CORT was shown to be an effective measure to differentiate between salamanders migrating over suitable/unsuitable substrates; however it was not an accurate predictor of other habitat attributes. CORT concentrations for salamanders collected in sub-standard habitats (with low proportion of canopy cover/swampy soil) were no different than in salamanders collected in ideal habitats (with high proportion of canopy/swampy soil) (Newcomb-Homan et al., 2003). The utilization of CORT as a measure of habitat quality may have limitations. The inability for CORT to accurately predict habitat attributes and quality may be a result of the rapid elevation of this hormone within only two minutes of capture and/or restraint (Romero & Reed, 2005). Obtaining baseline levels of CORT in wildlife species that are truly reflective of habitat disturbance can be difficult, and other methods of assessing the physiological consequences of habitat change on avian species should thus be investigated.

The first chapter of my thesis acts to provide the necessary background information for my research. I first review current knowledge of the biology, reproduction, and conservation status of lesser scaup (*Aythya affinis*), and describe the mechanisms of the stress response and impacts on the individual, reproduction, and future offspring. I also discuss the impacts of

human-induced habitat change and disturbance on wildlife species; finally I conclude with a brief preface to the utilization of biomarkers (including the emerging field of metabolomics).

1.2 An Introduction to the Study Species: The Lesser Scaup (*Aytha affinis*)

1.2.1 *General Biology and Behaviour: An Overview*

The lesser scaup (henceforth scaup; Figure 1.1) is a medium-sized diving duck that is endemic to most regions of North America with recorded breeding ranges in all of Canada except for New Brunswick, Nova Scotia, and Prince Edward Island (Austin, Cuter, & Afton, 1998). One of the latest fall migrants, the majority of scaup utilize the Mississippi or the Atlantic Flyway and over-winter on the Gulf and Atlantic Coasts (Austin et al., 1998).

Scaup are sexually dimorphic, with males ranging from 700 to 1200 g in mass, while females fall between 600 to 1100 g (considerable overlap may occur, particularly in the breeding season) (Austin et al., 1998). Males are easily distinguished in definitive alternate plumage by bright yellow eyes, a "slatty" blue bill; a black head, neck, and breast; white flanks and belly; and a white flecked gray/black back (Austin et al., 1998). In contrast, female scaup have bodies taking on various shades of brown, with white patches located just behind a dark gray bill (Austin et al., 1998). Female eye color can vary from an olive to a brownish-yellow color, and is indicative of maturity/age (Trauger, 1974). Scaup are commonly confused with the Greater Scaup (*Aythya marila*) due to similar plumage characteristics; and multiple field and wildlife studies group these two waterfowl species together (Austin et al., 2000).

Observations of scaup implanted with radio-transmitters on the wintering grounds in Indiana have suggested that feeding primarily occurs in ten-minute intervals (Custer, Custer, & Sparks, 1996). Main food sources consist of invertebrates such as amphipods, gastropods, coleopteran, *Hirudinea* and *Trichoptera* species (Bartonek & Hickey, 1969; Mitchell & Carlson, 1993).

1.2.2 *Breeding Ecology:*

There are three broad breeding areas for scaup; the boreal forest, the tundra, and the prairie parklands. Of these three biomes, the boreal forest supports the highest population of breeding scaup (68%) (Afton & Anderson, 2001), and will be the emphasis of this review unless stated otherwise.

Scaup are highly philopatric, with females returning to their hatch-place to breed and raise offspring of their own (Afton, 1984). Scaup typically pair *en route* to the breeding grounds during spring migration; with a majority of females paired by late spring (Austin et al., 1998). Arrival to the boreal forest can vary dramatically by region; but most often occurs in mid to late April (Austin et al., 1998). The scaup population sex ratio is disproportionately skewed with a high proportion of males compared to females, potentially leaving many males without mates during the breeding season. These unpaired scaup males have been observed displaying antagonistic behavior and harassment toward paired scaup (Hammel, 1973), while in comparison breeding pairs appear to be tolerant of each other and were not observed to defend fixed territories and/or areas (Hammel, 1973).

Female scaup in the prairie parklands search for ideal nesting sites 1-6 days prior to nest initiation (Austin et al., 1998). In the boreal forests of the Northwest Territories (NWT) female scaup appear to favour nesting sites with low tree cover and in the presence of breeding gulls and/or terns (family *Laridae*) (Fournier & Hines, 2001). Clutch initiation dates vary and span over 38 days, with incubation dates occurring in mid to late June (Afton, 1984; Brook, 2002). Older birds nest earlier than younger scaup (Afton, 1984). Mean clutch sizes for birds collected in the NWT varied from 7.8 (+/- 0.6 [SE]) to 10.52 (+/- 0.30) eggs/clutch (Brook, 2002; Fournier & Hines, 2001), with collected eggs being medium-sized with a mean volume of 46.34 cm^3 (Brook, 2002).

Scaup in the boreal forest can be victims of nest parasitism (a behaviour whereby a breeding hen lays her eggs in the nest of another bird), and are parasitized by multiple species including other female scaup, greater scaup, and northern pintail (*Anas acuta*) (Fournier & Hines, 2001). In the prairie pothole regions the number of parasitizing species also includes redheads (*Aythya americana)* and ruddy ducks (*Oxyura jamaicensis*) (Hammel, 1973). Nest losses are attributed to predators such as raccoons (*Procyon lotor*), red foxes (*Vulpes fulva*), striped skunks (*Mephitis mephitis*), mink (*Mustela vison*), weasels (*Mustela spp*.), crows (*Corvus brachyrhynchos*) and black-billed magpies (*Pica pica*) (Hammel, 1973). In cases where nesting failed, the probability of re-nesting was very low and varied from 0 (Hammel, 1973) to 16.4% (Afton, 1984).

1.2.3 *The Population Decline: Hypotheses and Theories*

The scaup has gained the attention of wildlife conservationists and biologists over the past few decades due to an unsteady, fluctuating population. Scaup in the boreal forest biomes have steadily declined at an estimated rate of 95 000 to 150 500 birds per year since the late 1970s (Afton & Anderson, 2001; Austin et al., 2006) (Figure 1.2) with populations failing to recover despite conservation efforts. In response to concerns over declining waterfowl populations, the North American Waterfowl Management Plan (NAWMP) was formed between Canada and the United States in the mid-1980s in order to more effectively manage wild waterfowl populations. Even with assistance from this initiative, population estimates of scaup from 2005 were still 46% below the long-term goals (Austin et al., 2006). As a popular game species sought after by many avid hunters in both central Canada and the United States (Austin et al., 1998), over-harvesting is a popular hypothesis among conservationists for the continued scaup population decline. A historical look at the continental scaup harvest for North America (including Canada and the United States) shows tremendous variability in the number of scaup harvested in the last thirty years, with an estimated low continental harvest of 170 591 birds in 1990 to a high of 798 861 birds in 1977 (Canadian Wildlife Service Waterfowl Committee, 2009). Attempts to correlate scaup population trends to annual harvest rates have noted that in the period between 1986-1994 when a marked decrease in the scaup population occurred, harvest was at one of the lowest rates (Afton & Anderson, 2001). These observations suggest that harvest alone was an unlikely cause for the continued population decline. However, other conservationists appear to disagree, and evidence supports the move towards lower harvesting rates. According to the Canadian Wildlife

Service's 2009 report on the Population Status of Migratory Game Birds in Canada; the continental harvest of scaup has decreased steadily over time (a total decrease of 61% between 1974 and 2008, respectively) potentially due to the decline in scaup abundance (Canadian Wildlife Service Waterfowl Committee, 2009). In June 2008 the United States Fish and Wildlife Service approved a harvest strategy that would potentially reduce the current scaup bag harvest limits from two birds to one for the Mississippi and Central Flyways. Opinions regarding the impact of harvest on scaup populations thus vary widely, even among experts in the field.

Exposure to contaminants and toxins has also proposed to be responsible for the scaup decline. However, scaup eggs collected in the boreal forest and prairie parkland of Canada and Alaska; concentrations of selenium (DeVink et al., 2008) DDE, mercury, and pesticides of scaup eggs were below minimum thresholds required to elicit negative embryotoxic effects or other effects in female scaup (Fox, MacCluskie, & Brook, 2005). However, while contaminant concentrations in scaup are not elevated enough to elicit lethal effects; sublethal effects might negatively impact the scaups' ability to reproduce. The diameter of seminiferous tubules in unpaired male scaup collected in the NWT boreal forest was negatively correlated with liver mercury concentration, when accompanied by high selenium concentrations (Pollock & Machin, 2009). Thus, contaminants appear to have some impact on scaup reproduction, and further study is required to assess the full effect of these substances.

Numerous other hypotheses have been proposed to explain the scaup decline. Lower female survival and recruitment have been proposed (Afton & Anderson, 2001; Brook & Clark, 2005), yet survival estimates of female scaup in the boreal forest over the breeding season (a period of 42 days) was estimated to be 0.8 (extrapolated from a weekly survival rate of 0.96) (Brook & Clark, 2005). These estimates were comparable to female scaup survival rates during

the summer months in the Prairie parkland regions, whereas 10-month survival probability was estimated at 0.7 and annual survival was estimated to be 0.58 (Rotella, Clark, & Afton, 2003). Considering that the nesting-phase is when scaup are at highest risk for predation (as they are confined to the nest during incubation) these survival rates appear quite high.

An increased male: female ratio, decreased offspring survival, decreased body condition during migration (the spring condition hypothesis), and modifications to food resources on the wintering/migration/breeding grounds (Afton & Anderson, 2001; Anteau & Afton, 2004; Austin et al., 2000; Austin et al., 2006; Fox et al., 2005) have also been proposed for contributing to the decreased scaup population. It is likely that multiple factors contribute or interact to negatively impact scaup. Efforts to determine the cause(s) for the population decline should thus continue in order to ensure the persistence of this waterfowl species.

1.3 The Stress Response: A Mechanism that Contributes to Survival

1.3.1 *The Stress Response: An Overview*

It is a common misconception that any environmental perturbation (such as changing of the seasons), extreme environments (such as deserts or the polar regions), or specific life history stages (migration or breeding) are stressful for individuals (Wingfield, 1994). If the event can be predicted prior to occurrence, then the individual should be behaviorally, physiologically, and/or morphologically adapted to the perturbation. For example, several avian species participate in the life-history stage of migration. These events are predictable and governed by environmental cues such as photoperiod or changes in temperature (Wingfield, Breuner, & Jacobs, 1997), and allow for the individual to anticipate changes and make required adjustments. Therefore, while such

events may appear to be unpredictable or may place higher energy demands on the individual (Wingfield et al., 1997)), they do not serve as truly stressful events (Wingfield, 1994).

The term "stressor" was originally defined as events that have the potential to alter homeostasis and/or physiology of the individual, and can be initiated through physical (e.g., food restriction (Janczak et al., 2007)) and/or behavioral changes (e.g., changes to population density (Sapolsky, 1992; Siegel, 1980; Wingfield et al., 1997; Wingfield & Ramenofsky, 1999). These stressors can be deleterious to the individuals' fitness if they are not successfully tolerated or evaded (Martin, 2009). Stressful events initiate the "stress response" (defined as behavioural and/or physiological changes that allow individuals to recover from negative conditions imposed by the stressor (Martin, 2009)). When the event is unpredictable, the resulting stress response (also termed the "emergency life history stage" (Martin, 2009)) can be categorized into four distinct phases (Wingfield et al., 1998):

1. *Deactivation of non-essential activities and/or behaviours:* An individual is faced with a stressor and abandons attempts at activities (e.g., mating) or behaviours (e.g., feeding) not immediately required for survival.

2. *Activation of the stress response:* The individual makes the decision to face the stressor, leave the stressor, or initially face the stressor then leave if conditions do not improve: If the individual decides to stay in an attempt to face the perturbation, a resulting stress response will ensue.

3. *Release of glucocorticoids and mobilization of energy reserves:* The individual experiences an increase in circulating blood glucose as a result of gluconeogenesis of protein stores, and lipolysis from lipid reserves (Sapolsky, Romero, & Munck., 2000).

4. *Termination and completion of the stress response:* The individual has either successfully coped with the stressor, or has been forced to abandon/flee the perturbation. When the stressor is removed in these instances the individual returns to the life history stage prior to the stressor occurring (Wingfield et al., 1998), and normal activities and/or behaviour resumes. All individuals strive to reach this end of physiological balance (Silverin, 1998; Wingfield et al., 1998).

The stress response is a complex interaction between the nervous system and endocrine system (Siegel, 1980), and is controlled by the hypothalamus-pituitary-adrenal (HPA) axis. When a stressor is initially perceived, stimulation of the hypothalamus causes corticotrophin-releasing-factor production, which stimulates the anterior pituitary gland to increase synthesis of adrenocorticotropin (ACTH) (Sapolsky et al.,1995). Transportation of ACTH then occurs via the blood to the adrenal gland cortex, where synthesis and release of glucocorticoids occur (Sapolsky, 1992; Siegel, 1980; Toates, 1995).

1.3.2 *The Potential Effects of Prolonged Stress on the Individual:*

The stress response is beneficial during acute stressors, where redirection of behaviour and physiology yields higher survival. For example, predator attacks are over in a span of

seconds to minutes; in which case the re-allocation of energy away from non-vital processes (such as grooming, feeding, mating behaviour, etc.) is favorable. Chronic (long-term, prolonged, or persistent) stressors, such as food deprivation, may take place over several days to months; where suppression of the aforementioned activities could serve highly detrimental to the individuals' fitness (measured as an individuals' genetic contribution to the population; usually through the provision of offspring and/or subsequent relatives) and/or survival. Very rarely in the natural world are chronic stressors observed without reprieve. Constantly elevated CORT is thus a consequence of a response designed for acute stressors (Wingfield et al., 1998).

The state of being in constant stress can undoubtedly negatively impact the individual. In the case of birds, chronic exposure to CORT may lead to protein loss, neuronal cell death (Wingfield, 1994), cardiovascular disease, hypercholesteremia, and gastrointestinal lesions (Siegel, 1980). Stress and CORT exposure can be highly detrimental to the individual over a long period of time, and may eventually impact reproduction and future-offspring if the stressor is not removed.

1.3.3 *Vertical Transmission of maternal CORT to offspring:*

Unlike most mammals, development of the chick occurs outside the parent, in an enclosed shell of an egg. Without the connection between offspring and parent, it is hypothesized that mothers deposit hormones into their eggs to ``communicate" to their offspring regarding present environmental conditions (Groothuis et al., 2005). For example, female European starlings (*Sturnus vulgaris*) in better body condition deposited less CORT into clutches, possibly indicating ideal environmental conditions to their young (Love, Bird, & Shutt, 2003). As might

be expected, the opposite occurs for avian species raised in suboptimal conditions. Japanese quail (*Coturnix coturnix japonica*) subjected to chronic restraint stress deposited significantly more CORT into follicles than control quail (Okuliarova et al., 2010). The eggs of female barn swallows (*Hirundo rustica*) exposed to a stuffed cat (a perceived predator) had increased CORT concentration compared to eggs of swallows not subjected to the stressor (Saino et al., 2005). Stress experienced by the mother may be transferred in the form of CORT to influence the development of offspring into manifesting characteristics that may optimize survival in suboptimal environments (Eriksen et al., 2003). Circulating maternal CORT levels are correlated with follicular CORT concentrations in European starlings (Love et al., 2008) and domestic chickens (Rettenbacher et al., 2005). Hormones in the eggs are maternal in origin as they are present in unfertilized eggs, and are present before the embryo is capable of endogenous secretion (Groothuis et al., 2005).

The precise mechanisms of how CORT is deposited into reproductive follicles is unknown, but evidence suggests that maternal CORT is reversibly bound to carrier proteins which, due to their lipophilic nature, are able to passively diffuse from the plasma into the follicle. CORT is then deposited along with the yolk in concentric layers to form the inside of the egg (Janczak, Braastad, & Bakken, 2006; Rettenbacher et al., 2005; Siegel, 1980).

1.3.4 *The Potential Effects of Chronic Stress on Reproduction and Offspring:*

Reproduction is an energetically expensive life stage that requires the caregiver to devote valuable time and resources towards the development and care of offspring. Chronically elevated circulating CORT may compromise immune system function during this period.

Nesting female common eiders (*Somateria mollissima*) implanted with CORT pellets had immunoglobulin levels nearly two times lower than eiders implanted with shams (Bourgeon, Le Maho, & Raclot, 2009). Regression of the thymus, spleen, and bursa of Fabricius resulting from depletion of lymphocytes can also occur with exposure to CORT (Siegel, 1980), and may alter white cell populations (increased heterophils and decreased lymphocytes) resulting in decreased resistance to viruses (Siegel, 1980).

Prolonged exposure to elevated CORT may interfere with an individual's ability to successfully reproduce; through stunting of gonadal organs, limiting the number of future progeny, or placing future-offspring at a disadvantage for survival by lowering egg quality or increased probability of embryo mortality. Long-term CORT exposure may cause decreased gonadal growth and size (Silverin, 1998) and may also decrease quantity and/or quality of the eggs laid. Female Japanese quail selected for high-stress response (i.e., treated with CORT-implants) had significantly reduced egg production compared to control females selected for the low-stress response (Satterlee, Cole, & Castille, 2007). Under chronic restraint-stress, female Japanese quail produced follicles and yolks of smaller mass (Okuliarova et al., 2010), and female quail selected for high-stress response had significantly lower mean egg fertility than low-stress females (Schmidt, Satterlee, & Treese, 2009). Long-term exposure to CORT may also negatively impact embryo survival *in vivo*. Female barn swallows, stressed by the presence of a perceived predator, had reduced egg hatchability compared to controls (Saino et al., 2005). Domestic chicken eggs injected with exogenous CORT had increased embryo mortality within the first 3 days of development (Eriksen et al., 2003; Janczak et al., 2006). Likewise, female Japanese quail selected for high-stress response treated with CORT-implants had significantly higher embryo mortality than any other treatment (Schmidt et al., 2009).

Prenatal CORT exposure can produce physical changes in offspring. These physical changes may place young birds at a disadvantage for survival. For example, barn swallow chicks exposed to CORT via injection prior to hatch had significantly shorter wing feathers than control chicks (Saino et al., 2005). These shorter wing feathers may prevent chicks from successfully fledging, or place greater energy requirements on the individual for flight. Domestic chicken (Janczak et al., 2006), black-legged kittiwake(*Rissa tridactyla*)(Lanctot et al., 2003), yellow-legged gull (*Larus michahellis*) (Rubolini et al., 2005), barn swallow (Saino et al., 2005), Japanese quail (Hayward & Wingfield, 2004), and zebra finch (*Taeniopygia guttata*)(Spencer & Verhulst, 2007) chicks exposed to high concentrations of CORT prior to hatch had decreased body mass compared to sham-inoculated and/or control eggs. Decreased growth and depressed skeletal development may occur in young birds exposed to long-term CORT in the egg (Saino et al., 2005; Siegel, 1980). These physical attributes may not only be permanent, and may negatively impact avian survival upon reaching adulthood.

Exposure to elevated CORT has also been shown to alter behaviour in young birds. For example, male domestic chicken chicks injected with CORT during the embryonic stage had increased foraging/eating time upon hatching (Janczak et al., 2007). In contrast offspring of white lohmann selected leghorn hens experimentally stressed through feed-restriction spent less time foraging and eating (Janczak et al., 2007). Contrasting effects of CORT are also seen in the ability of young chicks to communicate with their caregivers. Yellow-legged gull chicks exposed to CORT *in vivo* produced weaker, less frequent, vocalizations than controls, and had decreased begging rates for food than their siblings (Rubolini et al., 2005), while black-legged kittiwake chicks treated with CORT-implants begged more frequently for food than kittiwake chicks with sham implants (Kitaysky, Wingfield, & Piatt, 2001). The differing responses of offspring to

CORT may represent two different strategies of preparing for what is anticipated to be a stressful situation. Birds may increase time spent feeding/begging in order to store energy prior to encountering the stressor or they may reduce these behaviours, choosing instead to conserve the energy to face the stressor. Regardless of the strategy chosen to face the stressor, both behavioural and physiological changes may eventually negatively impact the individual.

Increases in maternal circulating CORT not only affects the chicks, but may also predispose the mother to participate in behaviours which will increase her own survival at the expense to her offspring. Adult pied flycatchers (*Ficedula hypoleuca)* treated with CORT implants ingested the majority of food they obtained, instead of providing it to their offspring (Silverin, 1998). European starlings with higher baseline plasma CORT concentrations were more prone to nest abandonment (Love et al., 2008). Black-legged kittiwake parents with CORT implants spent less time brooding/guarding chicks by making significantly more trips away from their nests compared to sham-implanted parents (Kitaysky et al., 2001). As most chicks are dependent on their parents for some aspect of their care (whether it be for protection from predators or the provision of food/warmth), premature departure of the parents reduces the likelihood of the individual successfully surviving into adulthood.

Exposure to CORT early in life may have negative impacts on the individual, even after the source of CORT has been removed or has ceased. Domestic chickens exposed to CORT *in vitro* had lower body mass at 11 weeks of age compared to controls (Eriksen et al., 2003). Zebra finch nestlings administered CORT orally were less able to compete and defend the use of a perch against control birds 50 days after treatment (Spencer & Verhulst, 2007). Exposure to stressors early in a young bird's life may also influence their personality and ability to cope with stressors. In a study monitoring the impact of restraint-induced stress on European white storks

(*Cicona cicona*) nestlings over a 5 year period; storks with low CORT survived and successfully entered the breeding population as recruits, while survival and recruitment probability were negatively correlated to storks with a high CORT (Blas et al., 2007). Storks that had low CORT were identified as having a "proactive" personality; characterized by bold, aggressive, or fast-explorer behaviour. In contrast, storks with high CORT were had a "reactive" personality characterized by shy, cooperative, or "slow-explorer" behaviour. It was hypothesized that, in cases where population density is high and food resources are plentiful, individuals with the proactive personality benefit, while those with the reactive personality are compromised by their inability to compete with conspecifics. Early exposure to CORT thus has many long-lasting effects on the individual; particularly for long-lived species such as the European Stork, that may influence survival rate and reproductive success.

Many theories exist for the evolutionary-retention of imprinting. It is a learning process that occurs early in life, whereby a newly-hatched chick forms a strong social preference designed to keep the young bird in close proximity to the parent and to help in recognition of conspecifics (Bateson, 1966; Bolhuis, Cook, & Horn, 2000; Bolhuis, 1991; Bolhuis, 1999; Horn, 2004; Nordgreen, Janczak, & Bakken, 2006). Occurrence in multiple avian classes suggests that this behaviour is extremely important for proper development and survival of young birds. The imprinting process can be impeded by CORT, as evidenced by the negative correlation between following behaviour and circulating plasma CORT (Bolhuis, 1991; Martin, 1978). Leghorn chicks hatched from eggs injected with CORT prior to incubation had lower preference scores for imprinted objects and spent significantly less time with the imprinting stimuli than control chicks injected with the sesame oil vehicle (Nordgreen et al., 2006). Without proper imprinting, young birds may become separated from their parents or fail to recognize potential breeding

partners. Thus, this process is important for survival and future fitness contributing to the population.

In summary, chronically elevated CORT may negatively impact an individual in a variety of ways; whether by altering the adult immune response, reproductive capability, or behavior in adults; or exerting deleterious physical or behavioural impacts on their offspring. While these effects may not be lethal; they may negatively impact an individual's ability to survive and successfully reproduce. Investigating sources of potential stressors in wildlife species may give insight into population declines; such as that of the lesser scaup.

1.4 Anthropogenic Disturbance: How Humans Change Habitat

1.4.1 *Habitat Change: An Introduction:*

Habitat has been defined as "*the resources and conditions present in an area that produce occupancy; including survival and reproduction by a given organism*" (Hall et al., 1997). Ideal habitat is unique for each species, and can be considered the sum of specific resources required by the individual for survival (Franklin, Noon, & George, 2002; Hall, Krausman, & Morrison, 1997). Anthropogenic (or human-induced) changes such as intensive agricultural development, forestry, mineral extraction, oil/gas industrial development, and road-building have negatively impacted multiple habitats including the boreal forests (Bayne & Hobson, 1998). These disturbances (defined as *any relative discrete event in time [natural or man-made] that disrupts the ecosystem, community, population structure and changes resources and/or substrate availability in the physical environment* (Hockin et al., 1992) have been known to cause i) reduced habitat or niche availability (known collectively as habitat loss) (Owens &

Bennett, 2000) and/or ii) habitat fragmentation (defined as the discontinuity of the environment resulting from any number of mechanisms that affect spatial distribution of resources that ultimately affect occupancy, reproduction, or survival for a specific species (Franklin et al., 2002)). Impacts of habitat fragmentation include i) edge effects (defined as the distinctive species composition and/or abundance found near the perimeter of an ecosystem) (Forman, 1995)) and/or ii) habitat isolation (defined as the distance of the observed habitat to neighboring habitats; or proportion of similar habitats within a certain area (Schmiegelow & Monkkonen, 2002). Distinguishing between habitat loss and fragmentation and their impacts on wildlife species can be extremely difficult given that both these phenomena often occur together (Fahrig, 1997), thus for the purposes of this review the term "habitat fragmentation" shall encompass the observed effects of habitat loss, edge effects, and habitat isolation.

1.4.2 *Direct and Indirect Impacts of Anthropogenic Disturbance on Wildlife Species*

Numerous studies have investigated the impact of anthropogenic habitat change on wildlife species (see reviews by Best et al., 1995; Brawn, Robinson, & Thomspon, 2001; Fry, 1995; Hockin et al., 1992; NRC, 2003; Reijnen & Foppen, 1997). Alterations can have direct impacts (as a direct result from disturbance; such as altered habitat availability or quality) and/or an indirect impact (usually a secondary effect caused by the direct effect; such as the alteration of foraging material and/or predator dynamics) (Truett, Miller, & Kertell, 1997).

The direct effects of anthropogenic disturbance are easier to observe than indirect effects, and may render a habitat unsuitable for wildlife species in a variety of ways. For example; farming practices on agricultural land may negatively affect breeding areas used by waterfowl

species by destroying nesting sites. In the prairie pothole regions of North Dakota, agricultural tillage activity was found to ruin 93% of all the active nests observed on cropland, for a total of destruction 34% of all duck nests observed (including nests found on non-tilled land) (Higgins, 1977). Use of toxic compounds can also directly affect wildlife species. Pesticides and fertilizers used on landscapes can persist in environments years after their use. For example, American robin (*Turdus migratorius*) eggs collected in orchards with a history of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) usage had significantly higher levels of DDT compared to non-orchard reference sites (Harris et al., 2000). The DDT concentration in robin eggs collected from orchards was comparable to that in field studies where mortality and reproductive effects ensued (Harris et al., 2000). Habitat displacement may also occur as a direct result of altered landscapes, as observed in California white-tailed kites actively avoid plowed fields, rural areas, and spaces of irrigated pastures throughout the year (Erichsen et al., 1996).

Indirect effects of anthropogenic disturbance are often difficult to assess. Most notable changes (as a result of fragmented landscapes) include an increased presence of predation in agricultural zones (Angelstam, 1986; Bayne & Hobson, 1997, 1998; Hartley & Hunter, 1998; Patten & Bolger, 2003; Zegers, May, & Goodrich, 2000), increased species turnover (Schmiegelow, Machtans, & Hannon, 1997), decreased species richness (Canaday, 1997; Tewksbury et al., 2002), altered species composition (Tewksbury et al., 2002), and increased brood parasitism (Bayne & Hobson, 2002). Other changes, such as decreased pairing and nest success (Bayne & Hobson, 2002) have also been noted in ovenbirds (*Seiurus aurocapillus*), with unsuccessful individuals leaving the fragmented areas in search of new territory.

Regardless of whether the habitat change directly or indirectly impacts wild species, it is made unsuitable in some way. Individuals may choose to avoid/leave the landscape (such as the

white-tailed kite and ovenbirds), or may choose to stay in the area and face the problems that come with habitat fragmentation. Both strategies require valuable time and energy on the individuals' part, potentially taking away from valuable behaviours/activities required for survival and/or breeding. The loss of habitat is more than the removal of pristine habitat; it may result in a cascade of effects that combine to negatively impact individuals and populations.

1.4.3 *Anthropogenically Altered Habitat and its Impact on Wildlife Species:*

The impacts of human-induced habitat change on wildlife species can extend beyond simple habitat loss. The removal of natural formations (such as trees and wetlands), along with the subsequent introduction and replacement of new structures (such as housing developments, roads, or oil-extraction equipment) and stimulants (such as new sights and sounds) may act as disturbances and/or stressors for species not accustomed to high human disturbance (and/or their activities) in close proximity.

Oil development and gas extraction is an intensive process which results in severe alterations to the landscape. Habitat alterations may be viewed as unfavourable to wildlife inhabiting the areas. Several waterfowl species avoid oil development sites (consisting of well pads and compressor stations) from distances ranging from 800m to 8000m (Barry, Spencer, & Canadian Wildlife Service, 1976; Bromley, 1985). Lesser scaup, in particular, had strong preferences for low-disturbance habitats, rather than sites in the vicinity of oil rigs (Barry et al., 1976). While there is some evidence that particular waterfowl species may eventually become accustomed to the oil-wells and compression stations (Barry et al., 1976), observations were limited to a few pairs which still maintained a wary distance from these new structures. True

habituation to these disturbances may not occur. Habitat change associated with oil and gas development has been shown to impact reproduction in an endangered species. Group counts of greater sage grouse (*Centrocercus urophosianus*) declined at a rate of 35% per year over a four year period (2001-2005) on coal-bed natural gas fields; and reproductive success declined if gas field development was within 3-5 km of a breeding population (Walker, Naugle, & Doherty, 2007). Agricultural practices and development may also negatively impact wildlife species. White stork nestlings (*Ciconia ciconia*) sampled in the vicinity of agricultural crop fields had baseline CORT levels nearly two times higher than storks sampled in low impact marsh areas (Blas et al., 2007), adding support to habitat alterations and changes acting as stressors. Overall, human-induced habitat change may have a variety of negative impacts on wildlife species, ranging from changes in behaviour, to eliciting stress responses and impacting reproduction for more sensitive species (Kurki et al., 2000).

The presence of humans and/or their recreational activities can alter behaviour for wildlife species. Staging canvasback ducks *(Aythya valisineria)* flushed more readily in relation to boating disturbance in spring than in the fall (Kahl, 1991). Common eider ducklings had increased clustering behaviour when faced with surfers or boating disturbance than when disturbed by shore-based activities (such as fishermen, cars, or pedestrian activity along the shoreline) (Keller, 1991). Eider ducklings also responded to these disturbances by spending increased time swimming, as opposed to ducklings from non-disturbed habitats that spent more time feeding and roosting (Keller, 1991). Capercaille (*Tetrao urogallus*) had higher fecal CORT levels in the ski season, and aggregated into smaller areas during the ski season than in the pre-ski season (Thiel et al., 2008). Southern rockhopper penguins (*Eudyptes chrysocome*

chrysocome) had higher yolk CORT concentrations in sites with human-exposure than rockhopper penguins from pristine sites with no exposure to humans (Poisbleau et al., 2009).

Suppression of non-essential activities in response to humans has also been observed. Breeding trumpeter swans (*Cygnus buccinator*) reduced feeding and preening time, and increased vigilance behaviour (head-up posture) in reaction to pedestrian disturbance (Henson & Grant, 1991). To the general public, these behavioural changes may not appear to significantly affect individuals; however, physiological impacts of disturbances may have deleterious effects on the animal, which may impact fitness and survival.

1.4.4 *Biomarkers: An Introduction*

Animals, including humans are complex organisms governed by a number of metabolic pathways, gene processes, and environmental factors (Nicholson, Holmes, & Lindon, 2007). With individual physiological regulation occurring at both the cellular (Nicholson et al., 2007) and systems levels; identifying a single measure to adequately reflect whole organism health is extremely difficult (if not impossible) due to the numerous ways health can be influenced by the environment.

Biomarkers are biochemical, physiological, or histological changes that occur as a result of exposure to chemicals, diseases, risk factors, or other stressors (Fox, 1993; Huggett et al., 1992; Kaddurah-Daouk et al., 2005). Biomarkers can come in various forms such as genetic markers, neuro-imaging biomarkers, clinical biomarkers, and/or biochemical markers (Kaddurah-Daouk et al., 2005), and can be extracted from physiological tissues or fluids (such as plasma or urine) which integrate the many biological processes (Nicholson et al., 2007). This

sample can then be analyzed for specific compounds providing information on the health status of the organism under study.

Information gathered from biomarkers can serve multiple purposes and have many applications; such as understanding progression of disease and subsequent evaluation of treatment plans in the biomedical sciences (Kaddurah-Daouk et al., 2005). Biomarkers may also serve as bio-indicators in the environmental sciences for instance, as a measure of the effects of exposure and impairment on organisms under certain conditions (Fox, 1993). In an area of research where studies are often observational in nature, the application of biomarkers to wildlife conservation and management research may have the potential to answer difficult questions regarding the organisms' physiological responses to changes in their habitat.

1.5 The Emerging Field of Metabolomics

The newly emerging field of metabolomics evaluates the occurrence of endogenous metabolites present in biological samples to make inferences about the physiological status of the individual. Metabolites are small molecules that arise during metabolism (Ramsden, 2009) or are the end products of cellular processes (Fiehn, 2002), and are the result of the interaction of the system's (or organism's) genetic make-up along with the numerous factors found in its environment (Rochfort, 2005). Examples of metabolites include, but are not limited to, carbohydrates, lipids, amino acids, organic acids, nucleotides, phosphagens, glycotic products, and Kreb cycle intermediates (Viant, Rosenblum, & Tjeerdema, 2003; Viant, 2007b).

The goal of metabolomic studies is to obtain an overall picture the organism's metabolic state at a particular moment in time, and to correlate the observed metabolites with an individual,

treatment, or a specific metabolic pathway (or alternatively; to demonstrate changes were not associated with these factors) (Kaddurah-Daouk et al., 2005). Metabolomic data can be compared between different groups of organisms in a variety of ways; such as controlled laboratory experiments (e.g., "treatment" vs. "control" contrasting alternative drugs or diets), across genetic lines/diversity (e.g., "genetic line 1" vs. "genetic line 2") or even amongst environmental conditions (e.g., "drought" vs. "non-drought") (Primrose & Twyman, 2006) in order to assess differences in metabolite composition (Kaddurah-Daouk et al., 2005). More complex studies have even compared metabolite profiles among multiple groups and across treatments; such as a study assessing the impact of captive and wild habitats on metabolite profiles of marine mussels (*Mytilus galloprovincialis*) placed in hypoxic and non-hypoxic environments (Hines et al., 2007).

Utilization of environmental metabolomics can offer some advantages in wildlife research, such as the small amount of biological sample required (Lacey et al., 1999; Viant, 2008), minimal sample preparation (Viant, 2007a), and quick/inexpensive sample analysis (Griffin & Shore, 2007; Samuelsson et al., 2006). Furthermore, metabolomic profiles generated are easily reproducible across different laboratories (Lenz et al., 2003; Nicholson et al., 2007; Viant et al., 2009).

The metabolome is comprised of all the metabolites present within a cell or individual at a specific moment in time (Fiehn, 2002; Miller, 2007; Rozen et al., 2005; Viant et al., 2003)). Acquisition of an organism's metabolome can be acquired through biofluids such as urine, blood serum or plasma (Beckonert et al., 2007). Cerebrospinal, seminal, amniotic digestive, blister, cyst, synovial, and dialysis fluids (in addition to lung aspirates and saliva) may also be utilized (Bollard et al., 2005; Nicholson & Wilson, 1989). These samples can be analyzed using any

number of analytical chemical techniques such as nuclear magnetic spectroscopy (NMR), mass spectroscopy (MS), gas/liquid chromatography (Fiehn, 2002), and the newer methods of ultra-high–pressure liquid chromatography (UPLC) (Nicholson et al., 2007). The resulting metabolic spectra (and patterns within) are known as "profiles" (Tomita, 2005) and can represent hundreds of different metabolites (Primrose & Twyman, 2006).

1.5.1 *Nuclear Magnetic Resonance Spectrometry*

Of all the analytical chemical methods developed and described for metabolite detection, only MS and NMR spectroscopy (elaborated below) have the capability of resolving large numbers of metabolites (Primrose & Twyman, 2006). While both NMR spectroscopy and MS have considerable value in metabolomics, NMR holds several advantages over alternative methods (including non-destructive preparation of biological samples, rapid analysis, and relatively low cost per-sample basis (Griffin & Shore, 2007; Primrose & Twyman, 2006; Samuelsson et al., 2006)).

One dimensional (or ^{1}H, also known as proton) NMR is the application of magnetic fields with respect to the naturally occurring hydrogen-1 nuclei found within the molecules of any substance (Ross et al., 2007). Hydrogen (in addition to having a charge and a respective mass) has a characteristic measure of angular momentum known as 'spin', which will vary according to the molecule under study. The presence of spin generates a magnetic moment, and the application of numerous magnetic fields by the NMR spectrometer takes advantage of this (Ross et al., 2007). In an NMR spectrometer, samples (biofluid or solid) are placed within a detection coil. An external magnetic field applied by the spectrometer causes the molecules

present in these samples to react by either aligning parallel ("up"), or anti-parallel ("down") as a result of their magnetic moments. Further application of additional magnetic fields at varying angles results in the particle "spinning" at a specific frequency, proportional to the applied magnetic field. The magnetization of the sample results in a specific voltage, which is picked-up and interpreted by the detection coil of the spectrometer (Ross et al., 2007).

After initial processing, the data are transformed into a spectral image of the metabolites present in the sample. The position of each spectral band (known as its chemical shift) is measured in parts-per-million (ppm) relative to that of an added reference substance; and provides valuable information as to the molecular group identity/substance (Nicholson et al., 2007). While many compounds are available, a reference standard often utilized is a variant of 3-trimethylsilylpropionic acid (TSP), with the respective methylene groups deuterated to avoid giving rise to peaks in the NMR spectrum (Nicholson et al., 2007).

1.5.2 *Environmental Metabolomics*

Environmental metabolomics is a newly emerging, yet fast advancing sub-discipline of the metabolomic field which evaluates the interaction organisms have with their environments (Griffin & Shore, 2007; Viant, 2008). The goal of environmental metabolomics is to characterize the metabolic state of free-living organisms (either observed in natural environments or raised in a laboratory settings that mimic the natural environment) (Morrison et al., 2007; Viant, 2007b). Environmental metabolomics has considerable potential for characterizing the responses of organisms to both natural and anthropogenic perturbations (Viant, 2008). Since this fields' emergence in the 1990s (Viant, 2008), environmental metabolomics has been successful in

uncovering metabolic differences between individuals exposed to physiological stressors including disease (Rozen et al., 2005; Stentiford et al., 2005; Viant et al., 2003), physical injury (Viant et al., 2005), temperature stress (Michaud & Denlinger, 2007), and the interaction between temperature and disease (Rosenblum, Tjeerdema, & Viant, 2006). Additional ecological stressors such as (but not limited to) desiccation, food availability, light/circadian rhythms, seasonality, atmospheric gases; as well as laboratory/field studies of eco- and environmental toxicology have also been studied using environmental metabolomics (see review by Bundy, Davey, & Viant, 2009).

The field of environmental metabolomics confers several advantages over alternative methods of assessing environmental health. First, environmental metabolomics does not attempt to predict which metabolites will be observed, or in what combinations and/or proportions they will appear. While this approach may seem counter-intuitive to those accustomed to generating *a priori* hypotheses, very little is known about the metabolic pathways associated with the stress-responses. Limiting the analyses may in fact result in the missed opportunity to identify new metabolites not previously known to participate in these pathways. This discovery-based approach results in a non-biased investigation (Fiehn, 2002; Lin, Viant, & Tjeerdema, 2006; Miller, 2007) where the researcher is granted the freedom to investigate any number of metabolites that may serve as important biomarkers for the stressor under study (Hines et al., 2007; Lin et al., 2006). This technique is unbiased and rapid, thus allowing metabolomics to be particularly powerful in discovering biomarker profiles (Viant, 2007b). Because metabolite profiles and data are limited to handful of study species (Miller, 2007), pre-defining which metabolites one hopes to find may actually hinder research as it automatically disqualifies other

metabolites which may be important biomarkers, but have not yet been discovered or identified (Lin et al., 2006).

1.6 Reproductive and Physiological Correlates of Habitat Disturbance:

Reproduction is an energetically expensive and complicated process which requires the mother to invest valuable time and resources. As reviewed earlier, habitat disturbance has been cited to negatively impact a variety of wildlife species in a variety of ways; ranging from changes in behaviour, destruction and loss of valuable breeding habitat, decreased reproductive performance, and induced physiological stress. While CORT exposure and elevation can certainly impact reproductive success of the individual, other aspects of breeding behaviour and ecology may impact both the mother as well as the offspring's chances of survival.

1.6.1 *Body Condition*

Of the many factors that have been proposed to negatively impact scaup reproductive success, body condition (defined as the relative size of energy stores to the structural size of the organism being studied (Green, 2001)) has been one of the most heavily researched. It has been hypothesized that a threshold amount of endogenous and exogenous nutrients for female scaup to partake in successful egg formation, clutch completion, and incubation; (Afton & Ankney, 1991) must first be accumulated.

The spring-condition hypothesis proposes that the reproductive capability of scaup is determined by the successful acquisition of nutrients during time spent on wintering, spring migration, and breeding grounds (Afton & Anderson, 2001) Protein and lipid stores seem to be particularly important, and appear to directly contribute to successful scaup reproduction. Female

scaup collected in Erikson, Manitoba were estimated to lose approximately 0.5 g of lipid for every 1 gram of lipid deposited during egg formation, and 0.1 g of mineral for each 1 gram of egg-shell produced (Afton & Ankney, 1991). As 68% of lipid reserves in scaup clutches collected from Alaska and Manitoba was of maternal origin, translating roughly into 27-47g of endogenous lipid reserve loss from of the mother (Esler, Grand, & Afton, 2001).

In a study of scaup collected in the central United States, both body mass and lipid reserves were observed to have negatively changed over time (Anteau & Afton, 2009). Specifically, mean lipid reserves were markedly lower (58-77g) when compared to female scaup collected in a stop-over area prior to migration (earlier in the season in the same year). Scaup collected in 2004/2005 were found to be in significantly worse body condition than females collected in 2001(Anteau & Afton, 2009). Stomach content samples of scaup collected from years 2004-2005 in Minnesota and Manitoba were 77 mg (49%) and 87 mg (52%) lighter than diet samples collected from scaup in the same location during the late 1970s and early 1980s (Anteau & Afton, 2008). These findings suggest that female scaup may have difficulties obtaining and maintaining sufficient nutrient reserves during migration. Consequently female scaup arriving on the breeding grounds in poor body condition (i.e., low protein and lipid stores) may be compromised in their ability to successfully reproduce.

Body condition is required not only for egg formation and clutch completion, but also for successful incubation of the eggs. As the mother is confined to the nest and has inadequate time to feed during the incubation period; sufficient nutrient stores must be accumulated prior to incubation. In a study assessing the impact of incubation body mass of the on nest success (defined as when a duckling hatched from and left the nest) for related species the Common Pochard (*Aythya ferina*) and the Tufted duck (*Aythya fuligula*), it was determined that incubating

females with successful nests were heavier than those that abandoned their nests for both species approaching the hatch date (Blums, Mednis, & Clark, 1997). Females of both species that were able to successfully hatch one duckling were significantly heavier than those females that failed in their attempt, and subsequently abandoned their nest (Blums et al., 1997). Incubating scaup collected in Erikson, Manitoba lost up to 5.33g/day in initial body mass over an estimated 26-day incubation period. At the conclusion of incubation it was estimated that female scaup would experience a total 20% reduction in body mass, or roughly 139 g in body weight (Afton & Ankney, 1991). Body mass of the incubating female is thus vital in ensuring successful incubation. Scaup average body condition has likely declined over time. In a study comparing the nutrient reserves from female scaup collected in Manitoba from the 1980s against those collected in 2000, it was calculated female scaup collected in the 1980s were (on average) 58.9 grams heavier than scaup collected from the 2000s. Explanations of the observed decrease in scaup body condition have centered primarily around the hypothesis that availability and quality of scaup food resources have been compromised. Hypothesized causes for the food decline have included the loss of semi-permanent and/or permanent wetlands used by scaup during spring migration (Austin et al., 1998), increased anthropogenic sedimentation (Anteau, 2002), use of agricultural chemicals that subsequently leach into wetlands (Tome, Grue, & Henry, 1995), and human disturbances (such as by recreational activities) that may reduce foraging time or increase the energy demands when waterfowl flee from the perceived stressors (Kahl, 1991).

1.6.2 *Nest Initiation Date*

Nest initiation date (the date on which the female lays the first egg in the clutch) is another strong determinant of successful reproduction. Scaup arriving on the breeding grounds in poor body condition must spend more time foraging in breeding areas before reaching the nutrition threshold required to develop eggs (Afton & Anderson, 2001). Given that scaup are relatively late in arriving on the breeding grounds compared to other waterfowl species, late arriving scaup could be delayed in nesting compared to other scaup arriving in better body condition (Esler et al., 2001). Average clutch size of scaup may decrease with the delay of nest initiation date because of seasonal decline in the average nutrient reserves (lipids, proteins, and minerals) required for egg production, (Esler et al., 2001). If a clutch is successfully completed, late nesters may face additional challenges in completing incubation. Female common pochard and tufted ducks that nested 3 days later were more likely to abandon their nests due to human disturbance and/or natural causes such as predation, than females that had initiated nests earlier in the breeding season (Blums et al., 1997). Ducklings hatched later in the breeding season have additional challenges. For example, mallard (*Anas platyrhynchos*) ducklings that hatched early in the season in east central Saskatchewan, were more likely to survive to 30 days of age than ducklings hatching later in the season (Dzus & Clark, 1998). The same trend was observed for common pochard and tufted duck ducklings, with survival and recruitment declining with advancing hatch date in Eastern Europe (Blums, Clark, & Mednis, 2002). Delayed nest initiation could impact breeding scaup at many stages of the reproduction cycle; reducing the number of potential recruits to the population. Nest initiation dates of scaup have changed recently from those historically recorded. For example, the average nest initiation date for female scaup

observed nesting near Yellowknife, Northwest Territories was June 19th and 21st in 1999 and 2000 respectively. In the same location during 1967-1970, the recorded nest initiation for scaup females was almost 2 weeks earlier, from June 7th to 13th (Brook, 2002).

Nest initiation in some non-waterfowl species can be impacted by neighboring anthropogenic disturbance. Greater sage grouse (*Centrocerus urophasianus*) using leks in the vicinity of gas development disturbances had a significantly reduced nest initiation rate of only 65% compared to grouse from undisturbed leks which had a nest initiation rate of 89% (Lyon & Anderson, 2003). As adult and yearling initiation rates were comparable at 71% and 69% respectively, nest initiation rates were viewed as reflective of the disturbance impacts felt by the grouse (Lyon & Anderson, 2003).

1.6.3 *The Link between Body Condition, Nest Initiation Date, and Habitat Quality*

Many factors are required for scaup to successfully reproduce, and many of these variables interact and are interdependent on each other. For example, it has been well established that body condition may impact nesting behaviour and success in several avian species. Nesting probability for wild female mallards observed across Canada were found to be positively correlated to body condition, while clutch size was found to be positively correlated with body condition and negatively correlated with nest initiation dates (Devries et al., 2009). Providing supplemental food to cooperative breeding Florida scrub-jays (*Aphelocoma coerulescens*)(Schoech, 1996) and Belted kingfishers (*Ceryle alcyon*) (Kelly & Van Home, 1997; Schoech, 1996) resulted in earlier initiated clutches for both species, and resulted in larger clutches for Florida scrub-jays compared to non-supplemented breeders.

Other aspects of habitat quality may also an important consideration for breeding waterfowl species. Nestboxes selected by Goldeneye (*Bucephala clangula*) females were found to be in the vicinity of large ponds than unused nestboxes, and usually closer to a water source than unused nests (Senechal, Gauthier,G, & Savard, 2008). Hence, what determines whether an individual will be reproductively successful is dependent on multiple, interacting aspects of reproductive biology in addition to habitat quality.

1.7 Conclusion:

In 1998, members of the first scaup workshop met to discuss priorities of research in order to determine causes for the continued scaup population decline. The most recent Scaup Workshop (held in January of 2006) generated a list of seven research priorities to aid scaup conservation efforts. Investigating "*habitat changes in the western boreal forest habitat affecting vital rates*" was listed as one of the highest priority topics. As discussed earlier; anthropogenic disturbances, including agricultural practices and industrial development have the potential to negatively impact wildlife species (both directly and indirectly). Continuous and ongoing disturbances may chronically stress individuals, and may adversely affect wildlife species through the suppression of seemingly non-essential behaviours or increasing susceptibility to certain diseases.

The second Scaup Workshop in 2006 also challenged researchers to assess the effectiveness of "other" methods (such as blood chemistry, radio-marking, or mark-re-sighting) in assessing breeding propensity of female scaup. Attempting to measure habitat disturbance through plasma CORT can be difficult due to rapid elevations in less than two minutes of capture (Romero & Reed, 2005). The use of other biomarkers as an assessment tool may be another

method scientists have been looking for in order to assess physiological changes that may be hampering female scaups' ability to successfully reproduce.

This research project combines two of the objectives recommended by members of the second Scaup Workshop by investigating the utilization of two potential biomarkers in relation to anthropogenic habitat change occurring in the boreal forest and female scaup reproduction. The conclusions drawn from these two projects could potentially provide insight as to underlying physiological changes accompanying habitat change, and its effect on reproducing female scaup in the boreal forest.

Figure 1.1:A group of Lesser Scaup (brown= females, black and white= males) near the St. Denis National Wildlife Area in Saskatchewan.

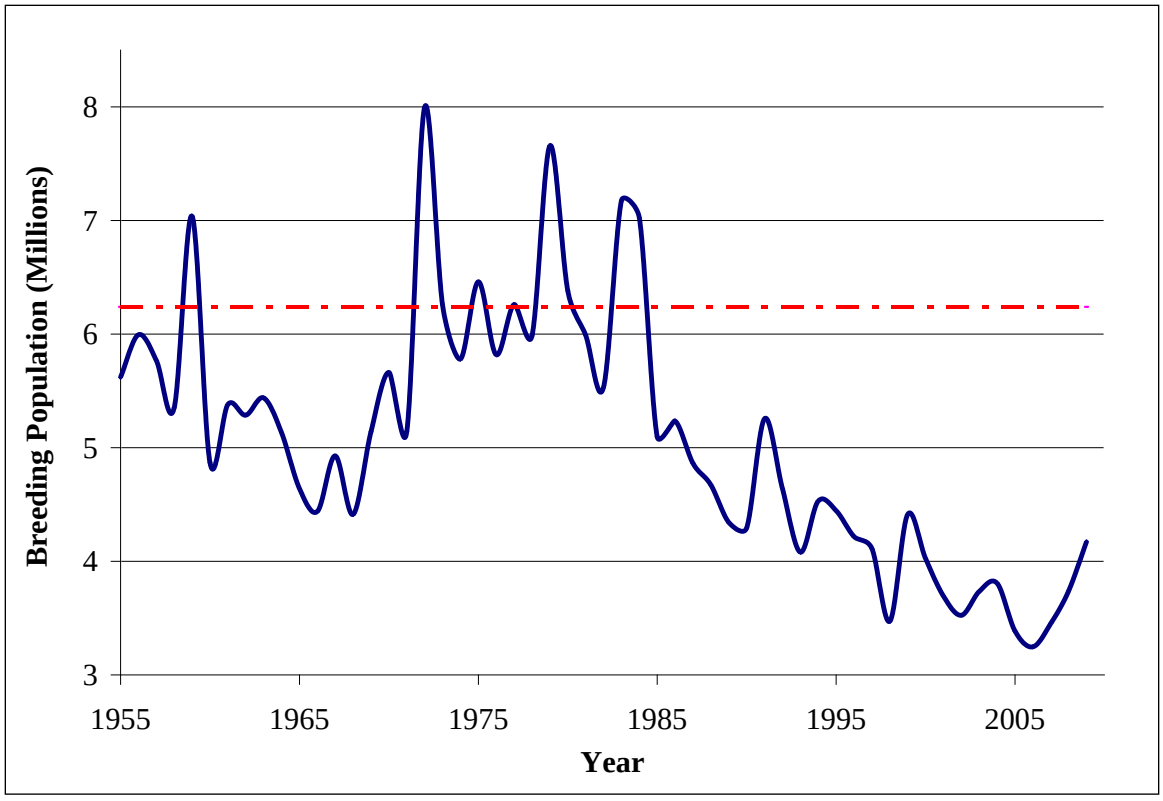

Figure 1.2: Long-term breeding population estimates (solid line) of Greater and Lesser Scaup in comparison to the North American Waterfowl Management Plan population goal (dashed line) in the traditional survey areas. Data obtained from U.S Fish & Wildlife Service "Trends in Duck Breeding populations, 1955-2009" report.

1.8 References

Afton, A. D. (1984). Influence of age and time on reproductive performance of female lesser scaup. *The Auk, 101*, 255-265.

Afton, A. D., & Anderson, M. G. (2001). Declining scaup populations: A retrospective analysis of long-term population and harvest survey data. *Journal of Wildlife Management, 65*(4), 781-796.

Afton, A. D., & Ankney, C. D. (1991). Nutrient-reserve dynamics of breeding lesser scaup: A test of competing hypotheses. *The Condor, 93*, 89-97.

Angelstam, P. (1986). Predation on ground-nesting birds' nests in relation to predator densities and habitat edge. *OIKOS, 47*, 365-373.

Anteau, M. J. (2002). Nutrient reserves of lesser scaup during spring migration in the Mississippi flyway: A test of the spring condition hypothesis. Unpublished MS Thesis, Louisiana State University, Baton Rouge, 1-51.

Anteau, M. J., & Afton, A. D. (2004). Nutrient reserves of lesser scaup (*Aythya affinis*) during spring migration in the mississippi flyway: A test of the spring condition hypothesis. *The Auk, 121*(3), 917-929.

Anteau, M. J., & Afton, A. D. (2008). Diets of lesser scaup during spring migration throughout the upper-midwest are consistent with the spring condition hypothesis. USGS Northern Prairie Wildlife Research Center, Paper. 12)

Anteau, M. J., & Afton, A. D. (2009). Lipid reserves of lesser scaup (*Aythya affinis*) migrating across the large landscape are consistent with the "spring condition" hypothesis. *The Auk, 126*(3), 873-883.

Austin, J. E., Afton, A. D., Anderson, M. G., Clark, R. G., Custer, C. M., Lawrence, J. S., Pollard, J.B., Ringelman, J.K. (2000). Declining scaup populations: Issues, hypotheses, and research needs. *Wildlife Society Bulletin, 28*(1), 254-263.

Austin, J. E., Anteau, M. J., Barclay, J. S., Boomer, G. S., Rohwer, F. C., & Slattery, S. M. (2006). Declining scaup populations: Reassessment of the issues, hypotheses, and research directions. *Second Scaup Workshop,* Bismarck, ND.

Austin, J. E., Custer, C. J., & Afton, A. D. (1998). Lesser Scaup(*Aythya affinis*). *The Birds of North America,* (338), 32.

Barry, T. W., Spencer, R., & Canadian Wildlife Service. (1976). *Wildlife response to oil well drilling*. Progress Notes. Ottawa: Canadian Wildlife Service.

Bartonek, J. C., & Hickey, J. J. (1969). Food habits of canvasbacks, redheads, and lesser scaup in Manitoba. *The Condor, 71*, 280-290.

Bateson, P. P. G. (1966). The characteristics and context of imprinting. *Biological Reviews, 41*, 177-220.

Bayne, E. M., & Hobson, K. A. (1997). Comparing the effects of landscape fragmentation by forestry and agriculture on predation of artificial nests. *Conservation Biology, 11*(6), 1418-1429.

Bayne, E. M., & Hobson, K. A. (1998). The effects of habitat fragmentation by forestry and agriculture on the abundance of small mammals in the southern boreal mixedwood forest. *Canadian Journal of Zoology, 76*, 62-69.

Bayne, E. M., & Hobson, K. A. (2002). Apparent survival of male ovenbirds in fragmented and forested boreal landscapes. *Ecology, 83*(5), 1307-1316.

Beckonert, O. K., Hector, C. B., Timothy, M. D., Bundy, J., Holmes, E., Lindon, J. C., Nicholson, J.K. (2007). Metabolic profiling, metabolomic and metabonomic procedures for NMR spectrosopy of urine, plasma, serum and tissue extracts. *Nature Protocols, 2*(11), 2692-2703.

Best, L. B., Freemark, K. E., Dinsmore, J. J., & Camp, M. (1995). A review and synthesis of habitat use by breeding birds in agricultural landscapes of Iowa. *American Midland Naturalist, 134*(1), 1-29.

Blas, J., Bortolotti, G. R., Tella, J. L., Baos, R., & Marchant, T. A. (2007). Stress response during development predicts fitness in a wild, long lived vertebrate. *Proceedings of the Royal Society of London, Series B, 104*(21), 8880-8884.

Blums, P., Clark, R. G., & Mednis, A. (2002). Patterns of reproductive effort and success in birds: Path analyses of long-term data from European ducks. *Journal of Animal Ecology, 71*, 280-295.

Blums, P., Mednis, A., & Clark, R. G. (1997). Effect of incubation body mass on reproductive success and survival of two european diving ducks: A test of the nutrient limitation hypothesis. *The Condor, 99*(4), 916-925.

Bolhuis, J. J., Cook, S., & Horn, F. (2000). Getting better all the time: Improving preference scores reflect increases in the strength of filial imprinting. *Animal Behavior, 59*, 1153-1159.

Bolhuis, J. J. (1991). Mechanisms of avian imprinting: A review. *Biological Reviews, 66*, 303-345.

Bolhuis, J. J. (1999). Early learning and the development of filial preferences in the chick. *Behavioural Brain Research, 98*, 245-252.

Bollard, M. E., Stanley, E. G., Lindon, J. C., Nicholson, J. K., & Holmes, E. (2005). NMR-based metabonomics approaches for evaluating physiological influences on biofluid composition. *NMR in Biomedicine, 18*, 143-162.

Bourgeon, S., Le Maho, Y., & Raclot, T. (2009). Proximate and ultimate mechanisms underlying immunosuppression during the incubation fast in female eiders: Roles of triiodothyronine and corticosterone. *General and Comparative Endocrinology, 163*, 77-82.

Brandt, C. A., Parrish, J. K., & Hodges, C. N. (1995). Predictive approaches to habitat quantification: Dark-dumped petrels on haleakala, maui. *The Auk, 112*(3), 571-579.

Brawn, J. D., Robinson, S. K., & Thomspon, F. R. (2001). The role of disturbance in the ecology and conservation of birds. *Annual Review of Ecology and Systematics, 31*, 251-276.

Bromley, M. (1985). Wildlife management implications of petroleum exploration and development in wildland environments No. Technical Report). Ogden,UT,USA: Forest Service.

Brook, R. W. (2002). Breeding ecology and local population dynamics of lesser scaup (*Aythya affinis*) in boreal forest of Western Canada. Unpublished MS Thesis, University of Saskatchewan, Saskatoon.

Brook, R. W., & Clark, R. G. (2005). Breeding season survival of female lesser scaup in the northern boreal forest. *Arctic, 58*(1), 16-20.

Bundy, J. G., Davey, M. P., & Viant, M. R. (2009). Environmental metabolomics: A critical review and future perspectives. *Metabolomics, 5*, 3-21.

Canaday, C. (1997). Loss of insectivorous birds along a gradient of human impact in amazonia. *Biological Conservation, 77*, 63-77.

Canadian Wildlife Service Waterfowl Committee. (2009). *Population status of migratory game birds in Canada, 2009* . CWS Migratory Birds Regulatory Report Number 28.

Custer, C. M., Custer, T. W., & Sparks, D. W. (1996). Radio telemetry documents 24-hour feeding activity of wintering lesser scaup. *Wilson Bulletin, 108*(3), 556-566.

DeVink, J. M., Clark, R. G., Slattery, S. M., & Trauger, D. L. (2008). Are late-spring boreal lesser scaup (*Aythya affinis*) in poor body condition? *The Auk, 125*(2), 291-298.

Devries, J. H., Brook, R. W., Howerter, D. W., & Anderson, M. G. (2009). Effects of spring body condition and age on reproduction in mallards (*Anas platyrhynchos*). *The Auk, 125*(3), 618-628.

Dzus, E. H., & Clark, R. G. (1998). Brood survival and recruitment of mallards in relaion to wetland density and hatching date. *The Auk, 115*(2), 311-318.

Erichsen, A. L., Smallwood, S. K., Commandatore, A. M., Wilson, B. W., & Fry, M. D. (1996). White-tailed kite movement and nesting patterns in an agricultural landscape. In D. M. Bird, D. Varland & J. Negro (Eds.), *Raptors in human landscapes: Adaptations to built and cultivated envrionments* (pp. 165). London: Academic Press Limited.

Eriksen, M. S., Haug, A., Torjesen, P. A., & Bakken, M. (2003). Prenatal exposure to corticosterone impairs embryonic development and increases fluctuating asymmetry in chickens (*Gallus gallus domesticus*). *British Poultry Science, 44*(5), 690-697.

Esler, D., Grand, J. B., & Afton, A. D. (2001). Intraspecific variation in nutrient reserve use during clutch formation by lesser scaup. *The Condor, 103*(4), 810-820.

Fagan, W. F., Cantrell, R. S., & Cosner, C. (1999). How habitat edges change species interactions. *The American Naturalist, 153*(2), 165-182.

Fahrig, L. (1997). Relative effects of habitat loss and fragmentation on population extinction. *Journal of Wildlife Management, 61*(3), 603-610.

Fiehn, O. (2002). Metabolomics- the link between genotypes and phenotypes. *Plant Molecular Biology, 48*, 155-171.

Forman, R. T. T. (1995). *Land mosaics: The ecology of landscapes and regions*. Cambridge: Cambridge University Press.

Fournier, M. A., & Hines, J. E. (2001). Breeding ecology of sympatric greater and lesser scaup (*Aythya marila* and *Aythya affinis*) in the subarctic northwest territories. *Arctic, 54*(4), 444-456.

Fox, G. A. (1993). What have biomarkers told us about the effects of contaminants on the health of fish-eating birds in the Great Lakes? The theory and a literature review. *Journal of Great Lakes Research, 19*(4), 722-736.

Fox, G. A., MacCluskie, M. C., & Brook, R. W. (2005). Are current contaminant concentrations in eggs and breeding female lesser scaup of concern? *The Condor, 107*, 50-61.

Franklin, A. B., Noon, B. R., & George, T. L. (2002). What is habitat fragmentation? *Studies in Avian Biology, 25*, 20-29.

Fry, D. M. (1995). Reproductive effects in birds exposed to pesticides and industrial chemicals. *Environmental Health Perspectives, 103*(7), 165-171.

Green, A. J. (2001). Mass/length residuals: Measures of body condition or generators of spurious results? *Ecology, 82*(5), 1473-1483.

Griffin, J. L., & Shore, R. F. (2007). Applications of metabonomics within environmental toxicology. In J. C. Lindon, J. K. Nicholson & E. Holmes (Eds.), *The handbook of metabonomics and metabolomics*. The Netherlands: Elsevier BV.

Groothuis, T. G. G., Muller, W., von Engelhardt, N., Carere, C., & Eising, C. (2005). Maternal hormones as a tool to adjust offspring phenotype in avian species. *Neuroscience and Biobehavioral Reviews, 29*, 392-352.

Hall, L. S., Krausman, P. R., & Morrison, M. L. (1997). The habitat concept and a plea for standard terminology. *Wildlife Society Bulletin, 25*, 171-182.

Hammel, G. S. (1973). The ecology of the lesser scaup (*Aythya affinis-eyton*) in southwestern Manitoba. Unpublished MS Thesis, University of Guelph, Guelph.

Harris, M. L., Wilson, L. K., Elliott, J. E., Bishop, A., Tomlin, A. D., & Henning, K. V. (2000). Transfer of DDT and metabolites from fruit orchard soils to American robins (*Turdus migratorius*) twenty years after agricultural use of DDT in Canada. *Archives of Environmental Contamination and Toxicology, 39*, 205-220.

Hartley, M. J., & Hunter, M. L. (1998). A meta-analysis of forest cover, edge effects, and artificial nest predation rates. *Conservation BIology, 12*(2), 465-469.

Hayward, L. S., & Wingfield, J. C. (2004). Maternal corticosterone is transferred to avian yolk and may alter offspring growth and adult phenotype. *General and Comparative Endocrinology, 135*, 365-371.

Henson, P., & Grant, T. A. (1991). The effects of human disturbance on trumpeter swan breeding behaviour. *Wildlife Society Bulletin, 19*, 248-257.

Higgins, K. F. (1977). Duck nesting in intensively farmed areas of North Dakota. *Journal of Wildlife Management, 41*(2), 232-242.

Hines, A., Oladiran, G. S., Bignell, J. P., Stentiford, G. D., & Viant, M. R. (2007). Direct sampling of organisms from the field and knowledge of their phenotype: Key reccomendations for environmental metabolomics. *Environmental Science & Technology, 41*, 3375-3381.

Hockin, D., Ounsted, M., Gorman, M., Hill, D., Keller, V., & Barker, M. A. (1992). Examination of the effects of disturbance on birds with reference to its importance in ecological assessments. *Journal of Environmental Management, 36*, 253-286.

Horn, G. (2004). Pathways of the past: The imprint of memory. *Nature Reviews, 5*, 108-120.

Huggett, R. J., Kimerle, R. A., Mehrle, P. M., Bergman, H. L., Dickson, K. L., Fava, J. A., et al. (1992). Introduction. In R. J. Huggett, R. A. Kimerle, P. M. Mehrle Jr. & H. L. Bergman (Eds.),

Biomarkers: Biochemical, physiological, and histological markers of anthropogenic stress. Michigan: Lewis Publishers.

Janczak, A. M., Braastad, B. O., & Bakken, M. (2006). Behavioral effects of embryonic exposure to corticosterone in chickens. *Applied Animal Behaviour Science, 96*, 69-82.

Janczak, A. M., Heikkila, M., Valros, A., Torjesen, P., Anderson, I. L., & Bakken, M. (2007). Effects of embryonic corticosterone exposure and post-hatch handling on tonic immobility and willingness to compete in chicks. *Applied Animal Behaviour Science, 107*, 275-286.

Janczak, A. M., Torjesen, P., Palme, R., & Bakken, M. (2007). Effects of stress in hens on behavior of their offspring. *Applied Animal Behaviour Science, 107*, 66-77.

Johnson, M. D. (2007). Measuring habitat quality: A review. *The Condor, 109*, 489-504.

Kaddurah-Daouk, R., Kristal, B. S., Bogdanov, M., Matson, W. R., & Beal, M. F. (2005). Metabolomics: A new approach towards identifying biomarkers and therapeutic targets in CNS disorders. In S. Vaidyanathan, G. G. Harrigan & R. Goodacre (Eds.), *Metabolome analyses: Strategies for systems biology* . New York, U.S.A: Springer Science and Business Media.

Kahl, R. (1991). Boating disturbance of canvasbacks during migration at Lake Poygan, Wisconsin. *Wildlife Society Bulletin, 19*, 242-248.

Keller, V. E. (1991). Effects of human disturbance on eider ducklings *Somateria mollissima* in an estuarine habitat in scotland. *Biological Conservation, 58*, 213-228.

Kelly, J. F., & Van Home, B. E. (1997). Effects of food supplementation on the timing of nest initiation in belted kingfishers. *Ecology, 78*, 2504-2511.

Kitaysky, A. S., Wingfield, J. C., & Piatt, J. F. (2001). Cortiosterone facilitates begging and affects resource allocation in the black-legged kittiwake. *Behavioral Ecology, 12*(5), 619-625.

Kurki, S., Nikula, A., Helle, P., & Linden, H. (2000). Landscape fragmentation and forest composition effects on grouse breeding success in boreal forests. *Ecology, 81*(7), 1985-1997.

Lacey, M. E., Subramanian, R., Olson, D. L., Webb, A. G., & Sweedler, J. V. (1999). High-resolution NMR spectroscopy of sample volumes from 1nL to 10 uL. *Chemical Reviews, 99*(10), 3133-3152.

Lanctot, R. B., Hatch, S. A., Gill, V. A., & Eens, M. (2003). Are corticosterone levels a good indicator of food availability and reproductive performance in a kittiwake colony? *Hormones and Behavior, 43*, 489-502.

Lenz, E. M., Bright, J., WIlson, I. D., Morgan, S. R., & Nash, A. F. P. (2003). A 1-H NMR-based metabonomic study of urine and plasma samples obtained from helathy human subjects. *Journal of Pharmaceutical and Biomedical Analysis, 33*, 1103-1115.

Lin, C. Y., Viant, M. R., & Tjeerdema, R. S. (2006). Metabolomics: Methodologies and applications in the environmental sciences. *Journal of Pesticide Science, 31*(3), 245-251.

Love, O. P., Bird, D. M., & Shutt, L. J. (2003). Plasma corticosterone in American kestrel siblings: Effects of age, hatching order, and hatching asynchrony. *Hormones and Behavior, 43*, 480-488.

Love, O. P., Wynne-Edwards, K. E., Bond, L., & Williams, T. D. (2008). Determinants of within- and among-clutch variation in yolk corticosterone in the European starling. *Hormones and Behavior, 53*, 104-111.

Lyon, A. G., & Anderson, S. H. (2003). Potential gas development impacts on sage grouse nest initiation and movement. *Wildlife Society Bulletin, 31*(2), 486-491.

Marra, P. P., & Holberton, R. L. (1998). Corticosterone levels as indicators of habitat quality: Effects of habitat segregation in a migratory bird during the non-breeding season. *Oecologia, 116*, 284-292.

Martin, J. T. (1978). Imprinting behaviour: Pituitary-adrenocorticoal modulation of the approach response. *Science, 200*, 565-567.

Martin, L. B. (2009). Stress and immunity in wild vertebrates: Timing is everything. *General and Comparative Endocrinology, 163*, 70-76.

Michaud, M. R., & Denlinger, D. L. (2007). Shifts in the carbohydrate, polyol, and amino acid pools during rapid cold-hardening and diapause-associated cold-hardening in flesh flies (*sarcophaga crassipalpis*): A metabolomic comparison. *Journal of Comparative Physiology B, 177*, 753-763.

Miller, M. G. (2007). Environmental metabolomics: A SWOT analysis (strengths, weaknesses, opportunities, and threats). *Journal of Proteome Research, 6*, 540-545.

Mitchell, C. A., & Carlson, J. (1993). Lesser scaup forage on zebra mussels at Cook nuclear plant, Michigan. *Journal of Field Ornithology, 64*(2), 219-222.

Morrison, N., Bearden, D., Bundy, J. G., Collette, T., Currie, F., Davey, M. P., et al. (2007). Standard reporting requirements for biological samples in metabolomics experiments: Environmental context. *Metabolomics, 3*, 203-210.

Newcomb-Homan, R., Regosin, J. V., Rodrigues, D. M., Reed, J. M., Windmiller, B. S., & Romero, L. M. (2003). Impacts of varying habitat quality on the physiological stress of spotted salamanders (*ambystoma maculatum*). *Animal Conservation, 6,* 11-18.

Nicholson, J. K., Holmes, E., & Lindon, J. C. (2007). Metabonomics and metabolomics techniques and their applications in mammalian systems. In Lindon J.C., Nicholson J.K. & E. Holmes (Eds.), *The handbook of metabonomics and metabolomics* . The Netherlands: Elsevier BV.

Nicholson, J. K., & Wilson, I. D. (1989). High resolution proton magnetic resonance spectroscopy of biological fluids. *Progress in NMR Spectroscopy, 21,* 449-501.

Nordgreen, J., Janczak, A. M., & Bakken, M. (2006). Effects of prenatal exposure to corticosterone on filial imprinting in the domestic chick, *gallus gallus domesticus* . *Animal Behaviour, 72,* 1217-1228.

National Research Council (2003). Cumulative environmental effects of oil and gas activities on alaska's north slope. Washington, D.C: National Academies Press.

Okuliarova, M., Sarnikova, B., Rettenbacher, S., Skrobanek, P., & Zeman, M. (2010). Yolk testosterone and corticosterone in hierarchical follicles and laid eggs of Japanese quail exposed to long-term restraint stress. *General and Comparative Endocrinology, 165,* 91-96.

Owens, I. P. F., & Bennett, P. M. (2000). Ecological basis of extinction risk in birds: Habitat loss versus human persecution and introduced predators. *PNAS, 97*(22), 12144-12148.

Patten, M. A., & Bolger, D. T. (2003). Variation in top-down control of avian reproductive success across a fragmentation gradient. *OIKOS, 101,* 479-488.

Poisbleau, M., Deongin, L., Trouve, C., & Quillfeldt, P. (2009). Maternal deposition of yolk corticosterone in clutches of southern rockhopper penguins (*Eudyptes chrysocome chrysocome*). *Hormones and Behavior, 55,* 500-506.

Pollock, B., & Machin, K. L. (2009). Corticosterone in relation to tissue cadmium, mercury and selenium concentrations and social status of male lesser scaup (*Aythya affinis*). *Ecotoxicology, 18,* 5.

Primrose, S. B., & Twyman, R. M. (2006). *Principles of gene manipulation and genomics* (7th ed.). Australia: Blackwell Publishing.

Ramsden, J. J. (2009). *Computational biology bioinformatics: An introduction* (2nd ed.). London: Springer-Verlag London Limited.

Reijnen, R., & Foppen, R. (1997). Disturbance by traffic of breeding birds: Evaluation of the effect and considerations in planning and managing road corridors. *Biodiversity and Conservation, 6,* 561-581.

Rettenbacher, S., Mostl, E., Hackl, R., & Palme, R. (2005). Corticosterone in chicken eggs. *Annals of the New York Academy of Sciences, 1046*, 193-203.

Rochfort, S. (2005). Metabolomics reviewed: A new "omics" platform technology for systems biology and implications for natural products research. *Journal of Natural Products, 68*, 1813-1820.

Romero, L. M., & Reed, J. M. (2005). Collecting baseline corticosterone samples in the field: Is under 3 min good enough? *Comparative Biochemistry and Physiology, Part A*, 140, 73-79.

Rosenblum, E. S., Tjeerdema, R. S., & Viant, M. R. (2006). Effects of temperature on host-pathogen-drug interactions in red abalone, *Haliotis rufescens*, determined by 1H-NMR metabonomics. *Environmental Science and Technology, 40*, 7077-7084.

Ross, A., Schlotterbeck, G., Dieterle, F., & Senn, H. (2007). NMR spectroscopy techniques for application to metabolomics. In J. C. Lindon, J. K. Nicholson & E. Holmes (Eds.), *The handbook of metabonomics and metabolomics* . The Netherlands: Elsevier BV.

Rotella, J. J., Clark, R. G., & Afton, A. D. (2003). Survival of female lesser scaup: Effects of body size, age, and reproductive effort. *The Condor, 105*, 336.

Rozen, S., Cudkowicz, M. E., Bogdanov, N., Matson, W. R., Kristal, B. S., Beecher, C., Harrison, S., Vouros, P., Flarakos, J., Vigneau-Callahan, K., Matson, T., Newhall, K.M., Beal, M.F., Brown, R.H.Jr., Kadurrah-Daouk, R. (2005). Metabolomic analysis and signatures in motor neuron disease. *Metabolomics, 1*(2), 101-108.

Rubolini, D., Romano, M., Boncoraglio, G., Ferrari, R. P., Martinelli, R., Galeotti, P., Fasola, M., Saino, N.(2005). Effects of elevated egg corticosterone levels on behavior, growth, and immunity of yellow-legged gull (*larus michahellis*) chicks. *Hormones and Behavior, 47*, 592-605.

Saino, N., Romano, M., Ferrari, R. P., Martinelli, R., & Moller, A. O. (2005). Stressed mothers lay eggs with high corticosterone levels produce low-quality offspring. *Journal of ExperimentalZoology, 303*(A), 998-1006.

Samuelsson, L. M., Forline, L., Karlsson, G., Adolfsson-Erici, M., & Joakim Larsson, D. G. (2006). Using NMR metabolomics to identify responses of an environmental esterogen in blood plasma of fish. *Aquatic Toxicology, 78*, 341-349.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews, 21*(1), 55-89.

Sapolsky, R. M. (1992). Neuroendocrinology of the stress-response. In J. B. Becker, S. M. Breedlove & D. Crews (Eds.), *Behavioral endocrinology* . Massachusetts: Massachusetts Institute of Technology.

Satterlee, D. G., Cole, C. A., & Castille, S. A. (2007). Maternal corticosterone further reduces the reproductive function of male offspring hatched from eggs laid by quail hens selected for exaggerated adrenocortical stress responsiveness. *Poultry Science, 86*, 572-581.

Schmidt, J. B., Satterlee, D. G., & Treese, S. M. (2009). Maternal corticosterone reduces egg fertility and hatchability and increases the numbers of early dead embryos in eggs laid by quail hens selected for exaggerated adrenocortical stress responsiveness. *Poultry Science, 88*, 1352-1357.

Schmiegelow, F. K., Machtans, C. S., & Hannon, S. J. (1997). Are boreal birds resilient to forest fragmentation? An experimental study of short-term community responses. *Ecology, 78*(6), 1914-1932.

Schmiegelow, F. K., & Monkkonen, M. (2002). Habitat loss and fragmentation in dynamic landscapes: Avian perspectives from the boreal forest. *Ecological Applications, 12*(2), 375-389.

Schoech, S. J. (1996). The effect of supplemental food on body condition and the timing of reproduction in a cooperative breeder, the Florida scrub-jay *The Condor, 98*, 234-244.

Senechal, H., & Gauthier,G., Savard,J.P.L. (2008). Nesting ecology of common goldeneyes and hooded mergansers in a boreal river system. *The Wilson Journal of Ornithology, 120*(4), 732-742.

Siegel, H. S. (1980). Physiological stress in birds. *BioScience, 30*(8), 529-534.

Silverin, B. (1998). Stress responses in birds. *Poultry and Avian Biology Reviews, 9*(4), 153-168.

Spencer, K. A., & Verhulst, S. (2007). Delayed behavioral effects of postnatal exposure to corticosterone in the zebra finch (*taeniopygia guttata*). *Hormones and Behavior, 51*, 273-280.

Stentiford, G. D., Viant, M. R., Ward, D. G., Johnson, P. J., Martin, A., Wenbin, W., et al. (2005). Liver tumors in wild flatfish: A histopathological, proteomic, and metabolomic study. *OMICS: A Journal of Integrative Biology, 9*(3), 281-299.

Tewksbury, J. J., Black, A. E., Nur, N., Saab, V. A., Logan, B. D., & Dobkin, D. S. (2002). Effects of anthropogenic fragmentation and livestock grazing on western riparian bird communities. *Studies in Avian Biology, 25*, 158-202.

Thiel, D., Jenni-Eiermann, S. J., Braunisch, V., Palme, R., & Jenni, L. (2008). Ski tourism affects habitat use and evokes a physiological stress response in capercaille *tetrao urogallus*: A new methodological approach. *Applied Ecology, 45*, 845-853.

Toates, F. (1995). *Stress: Conceptual and biological aspects*. West Sussex: John Wiley & Sons Ltd.

Tome, M. W., Grue, C. E., & Henry, M. G. (1995). Case studies: Effects of agricultural pesticides on waterfowl and prairie pothole wetlands. In D. J. Hoffman, B. A. Rattner & Burton,G.A.Jr, Caims,J.Jr (Eds.), *Handbook of ecotoxicology* (pp. 565). Florida: Lewis Publishers.

Tomita, M. (2005). Overview. In M. Tomita, & T. Nishioka (Eds.), *Metabolomics: The frontier of systems biology* . Tokyo, Springer-Verlag.

Trauger, D. L. (1974). Eye color of female lesser scaup in relation to age. *The Auk, 91*(2), 243-354.

Truett, J. C., Miller, M. E., & Kertell, K. (1997). Effects of arctic alaska oil development on brant and snow geese. *Arctic, 50*(2), 138-146.

Viant, M. R., Bearden, D. W., Bundy, J. G., Burton, I. W., Collette, T. W., Ekman, D. R., et al. (2009). International NMR-based environmental metabolomics intercomparison exercise. *Environmental Science & Technology, 43*, 219-225.

Viant, M. R., Lyeth, B. G., Miller, M. G., & Berman, R. F. (2005). An NMR metabolomic investigation of early metabolic disturbances following traumatic brain injury in a mammalian model. *NMR in Biomedicine, 18*, 507-516.

Viant, M. R., Rosenblum, E. S., & Tjeerdema, R. S. (2003). NMR-based metabolomics: A powerful approach for characterizing the effects of environmental stressors on organism health. *Environmental Science Technology, 37*, 4982-4989.

Viant, M. R. (2007a). Environmental metabolomics using 1H-NMR spectroscopy. In C. C. Martin (Ed.), *Environmental genomics* (pp. 137-150). Totowa, NJ: Humana Press.

Viant, M. R. (2007b). Metabolomics of aquatic organisms: The new 'omics' on the block. *Marine Ecology Progress Series, 332*, 301-306.

Viant, M. R. (2008). Recent developments in environmental metabolomics. *Molecular Biosystems, 4*, 980-986.

Walker, B. L., Naugle, D. E., & Doherty, K. E. (2007). Greater sage-grouse population response to energy development and habitat loss. *The Journal of Wildlife Management, 71(8)*, 2644-2654.

Whittingham, M. J., Swetnam, R. D., Wilson, J. D., Chamberlin, D. E., & Freckleton, R. P. (2005). Habitat selection by yellowhammers *Emberiza citrinella* on lowland farmland at two spatial scales: Implications for conservation management. *Journal of Applied Ecology, 42*, 270-280.

Wingfield, C. (1994). Modulation of the adrenocortical response to stress in birds. In G. Davey, E. Peter & S. S. Tobe (Eds.), *Perspectives in comparative endocrinology* (pp. 520-528). Ottawa: National Research Council of Canada.

Wingfield, J. C., Breuner, C., & Jacobs, J. (1997). Corticosterone and behavioral responses to unpredictable events. In S. Harvey, & R. J. Etches (Eds.), *Perspectives in avian endocrinology* (pp. 267-278). Bristol, UK: Journal of Endocrinology Limited.

Wingfield, J. C., Maney, D. L., Breuner, C. W., Jacobs, J. D., Lynn, S., Ramenofsky, M., et al. (1998). Ecological bases of hormone-behavior interactions: The "emergency life history stage". *American Zoologist, 38*, 191-206.

Wingfield, J. C., & Ramenofsky, M. (1999). Hormones and the behavioral ecology of stress. In P. H. M. Balm (Ed.), *Stress physiology in animals* (pp. 1-44). Sheffield, England: Sheffield Academic Press.

Zegers, D. A., May, S., & Goodrich, L. J. (2000). Identification of nest predation at farm/forest edge and forest interior sites. *Journal of Field Ornithology, 71*(2), 207-216.

2 FOLLICULAR CORTICOSTERONE, BODY MASS, AND NEST-INITIATION DATE AS MEASURES OF HABITAT DISTURBANCE IN NESTING FEMALE LESSER SCAUP

2.1 Introduction:

In North America, lesser scaup (*Aythya affinis*, henceforth scaup) populations declined sharply during the 1980s, and have remained relatively stable in the past 15 years (Austin et al., 2000; Austin et al., 2006; Zimpfer et al., 2009). While populations rebounded in the last 3-5 years, the scaup population estimate from the 2012 spring season was near the long-term average (Zimpfer et al., 2009) yet below the goal set by the North American Waterfowl Management Plan (Austin et al., 2006). With 67% of the breeding population nesting in the boreal forests of Canada (Afton & Anderson, 2001), understanding factors that may impact reproduction is vital to the successful development and implementation of wildlife management decisions.

Female scaup are highly philopatric and return to the vicinity of their previous breeding location to breed and raise young (Afton, 1984; Johnson & Grier, 1988). Philopatry is an ideal strategy when individuals are born in high quality habitats and may aid in improving reproductive success (Kokko & Sutherland, 2001). However, anthropogenic activities can significantly alter the boreal forest landscape (Bayne & Hobson, 1998) and result in degradation of optimal breeding habitat. Scaup are medium-sized diving ducks that feed heavily on benthic and pelagic macroinverbrates. Nesting female scaup favour habitats with fresh or slightly brackish seasonal and semi-permanent wetlands with bulrush (*Scirpus* sp.) and cattail (*Typha* sp.) vegetation (Austin, Cuter, & Afton, 1998), and may overlook potential disturbances in upland habitats situated near these preferred habitats (see (Barry, Spencer, & Canadian Wildlife Service,

1976; Blas et al., 2007; Bromley, 1985). Decisions made by individuals to nest in areas using environmental cues which may have signified high habitat quality in the past, may then lead to failed nesting due to anthropogenic disturbances. These are known as evolutionary traps (see Schlaepher, Runge, & Sherman (2002) for review). Nesting scaup may be experiencing this phenomenon, and hence may be situated in altered landscapes among man-made structures (such as roads, buildings, agricultural disturbance, oil well pads, etc.) causing immediate and/or chronic stress.

Previous studies indicate that anthropogenic habitat disturbance may alter natural behaviours exhibited by waterfowl species (Barry et al., 1976; Bromley, 1985; Kahl, 1991; Keller, 1991), and may possibly be increasing stress. When stressed, birds release the glucocorticoid corticosterone (CORT); which mediates physiological and behavioural changes to assist the individual in coping with the stressor. While short-term CORT exposure is beneficial and may increase survival, chronic (or long-term) exposure to CORT may have several detrimental effects on the individual including altered energy metabolism (Siegel & Van Kampen, 1984), decreased immune function (Bourgeon, Le Maho, & Raclot, 2009), and protein loss (Wingfield, 1994). Chronic exposure to CORT may also have impacts on offspring, as passive maternal transfer of CORT into reproductive follicles occurs (Janczak, Braastad, & Bakken, 2006; Rettenbacher et al., 2005; 21Siegel, 1980). Young birds exposed to CORT during development may experience poor survival due to behavioural changes, including reduced ability to communicate with their parents (Kitaysky, Wingfield, & Piatt, 2001; Rubolini et al., 2005) and forage for food (Janczak et al., 2007). Barn swallow (*Hirundo rustica*) chicks hatched from eggs injected with CORT hatched with severely altered physical characteristics, including decreased growth and altered skeletal development (Saino et al., 2005).

Body condition is an important determinant of reproductive success. According to the parental quality hypothesis, females of higher quality may exhibit differential physiological and/or behavioural attributes which may confer advantages by being more reproductively successful than females in poorer condition (Gurney, Clark, & Slattery, 2012). Female body mass was found to be an important contributing factor to duckling survival rate for scaup in the Northwest Territories, with a seasonal decline in duckling survival rate for light-weight females (Gurney et al., 2012). Similarly, common goldeneye (*Bucephala clangula*) females in better body condition were more likely to successfully rear their ducklings to the first-week of age than to females in poorer condition (Paasivaara & Poysa, 2007). It is possible that females in poorer body condition may temporarily leave their brood in order to replenish lipid reserves that have been depleted as a result of the breeding season, resulting in inadequate parental care of offspring (Talent, Jarvis, & Krapu, 1983).

The spring condition hypothesis proposes that the reproductive capability of scaup is determined by successful acquisition of nutrients during wintering, spring migration, and/or on the breeding grounds (Afton & Anderson, 2001). Endogenous and exogenous nutrients stores are critical for production of nutrient-rich yolk, important for duckling development (Afton & Ankney, 1991). Sufficient body mass and energy stores are essential for clutch size, brooding and for parental care. Incubating scaup in Erikson, Manitoba lost a total of 20% body mass (approximately 139 g) during the incubation stage (Afton & Ankney, 1991). In a study assessing the reproductive success of common pochard (*Aythya farina*) and tufted duck (*Aythya fuligula*), females that were able to successfully rear one duckling to adulthood were heavier in body mass than females with unsuccessful nests (Blums, Mednis, & Clark, 1997). Hence, body mass is a strong determinant of whether or not a female will produce recruits into the population.

Methods of determining an index of body condition have utilized body mass, adjusted accordingly incorporating one (or more) measurements of structural body size. In my study, principal component analysis (PCA) analysis utilizing culmen length (body measurement most highly correlated to body mass) accounted for 43% of variation observed. However, there have been calls for scrutiny on the use of unverified indices of body conditions in these studies (Schamber, Esler & Flint, 2009). An analysis evaluating the ability of various conditions of body indices to estimate the amount of protein and lipid stores in lesser scaup determined body mass was the best predictor of nutrient stores, better than condition indices accounting for structural size (Schamber et al, 2009). A similar finding found fresh body mass explained 81% of variation in total lipids observed in lesser scaup, but incorporation of structural size only resulted in a 1% increase in explanatory power (Chappel & Titman, 1981).. Thus in my study, fresh body mass was used as I felt it most accurately provided insight into female body condition.

Nest initiation date (NID), defined as the date when the female lays the first egg in the clutch, also affects waterfowl reproductive success. In a study assessing impact of growing season length (time lapsed between first freeze and spring thaw of soils) on NID in scaup, initiation dates were unrelated to growing season length (Gurney et al., 2011). However, the flexible nesting date hypothesis proposes that females may alter breeding behaviour to correspond with environmental cues and conditions in order to increase reproductive success (Gurney et al., 2011; Visser et al., 1998). If this is the case, then scaup nesting in the vicinity of habitat disturbances may alter timing of breeding and, thus, reproductive behaviour in ways that may be detrimental.

Nesting later in the breeding season may be disadvantageous for several reasons. Seasonal decline in nutrient reserves required for egg formation may result in delayed nest

initiation with smaller clutches, compared to scaup that nested earlier (Esler, Grand, & Afton, 2001; Gurney et al., 2011), limiting offspring production. However, scaup ducklings at the St. Denis Wildlife National Wildlife Area in Saskatchewan originating from larger eggs that hatched later in the breeding season had greater probability of survival than scaup ducklings originating from smaller eggs , or those laid earlier in the season (Dawson & Clark, 1996). Scaup duckling survival may thus depend on many factors outside of nest initiation date. Ducklings from late-initiated nests may also have lower survival compared to ducklings hatched earlier in the season (Blums et al., 2005). However, earlier hatched scaup ducklings had higher age-correlated mass and longer head and culmen lengths compared to their later-hatched counterparts (Dawson & Clark, 2000). Negative effects of delayed NID may also impact future generations. Scaup ducklings hatched later in the season were less likely to be recruited than ducklings that had hatched earlier in the season (Dawson & Clark, 2000). This may be related to the additional time earlier-hatched ducklings have to become adept at flying and gain enough energy stores for migration (Dawson & Clark, 2000). An increased probability of nest-abandonment has been observed among waterfowl species such as the common pochard and tufted duck that nested later in the season (Blums et al., 1997). The impact of delayed NID on all these factors may ultimately determine duckling survival and recruitment.

Conventional methods of evaluating the impact of habitat disturbance on avian species have involved measuring circulating levels of CORT in the blood (Thiel et al., 2008; Wasser et al., 1997). However, this method only provides insight into the acute stress experienced by the individual and does not provide insight into the impact habitat disturbances may have on reproductive success. As reproductive follicles take (on-average) 5-6 days between formation and ovulation (Batt et al., 1992; Esler, 1994), ovarian corticosterone (OCORT) levels may

provide a means to assess long-term stress. Therefore, OCORT may provide insight into the accumulation of environmental cues and negative consequences of habitat disturbance. Both NID and body mass vary in response to habitat disturbances (Burton et al., 2006; Lyon & Anderson, 2003; Mainguy et al., 2002). Assessing how these variables change in relation to habitat quality may provide new insights regarding the impact of anthropogenic activities on nesting scaup.

The aim of my study was to investigate habitat attributes (either anthropogenic disturbance and/or natural landscape features) that have the greatest influence on OCORT, body mass, and NID of female scaup breeding in the Northern boreal forest in Alberta. I hypothesize that scaup collected in areas of high anthropogenic disturbance will display higher OCORT, while scaup breeding in areas of low anthropogenic disturbance and in the presence of high-quality boreal forest habitat will have lower OCORT amounts. Female scaup may avoid anthropogenic disturbances (see Kahl, 1991), and anthropogenic changes to the landscape may limit the access to suitable nesting habitat. I hypothesize that scaup nesting in habitats with a high cumulative proportion of man-made disturbance will delay nest initiation date and body mass. Similarly man-made disturbances might disrupt foraging for food as found for trumpeter swans (Henson & Grant, 1991), and may limit the availability of food resources through removal of suitable wetland habitats or through the use of industrial/agricultural products. As such, I predict that scaup collected in areas of high human disturbance will have lower body mass compared to scaup from areas of low anthropogenic habitat disturbance.

2.2 Methods:

2.2.1 *Field Collection:*

The study area encompassed the boreal forest fringes and areas of anthropogenic disturbance located in Northern-central Alberta in and between the general vicinities of Utikuma Lake (55° 52′ 0″ N, 115° 25′ 0″ W) and east towards Lac La Biche (54° 46′ 7″ N, 111° 58′ 50″ W) (Figure 2.1). Birds were collected from June 7th-13th and June 6th-14th (early-laying period) in 2008 and 2009, respectively. Scaup population and wetland abundance data (unpublished data, Mark Bidwell) and habitat layers obtained from IHS Incorporated (Englewood, Colorado, United States of America) and Duck's Unlimited Canada (Stonewall, Manitoba, Canada) were used to prioritize areas of collection based on documented high and low human-disturbance in the 2008 field season. In 2009, the collection strategy was refined by selecting areas of high and low agriculture/oil impact, or low anthropogenic habitat-disturbance areas on 10x10 kilometre grids of habitat data layers.

Female scaup were collected by shooting individuals from groups of less than four scaup (including pairs) being targeted by teams of experienced hunters in order to reduce probability of accidental sampling from migrating scaup (DeVink et al., 2008). This study was approved by the University of Saskatchewan Animal Care and Use Program, in accordance with the Canadian Council on Animal Care and guidelines approved protocols. In the 2008 field season, female scaup were first collected in the general vicinity of Athabasca, then west towards Slave Lake. Due to the lack of ovarian development, and hence suitable ovaries collected from scaup residing in areas of high agricultural disturbance (from the region surrounding Athabasca), samples collected in the 2008 field season were biased towards areas with oil and gas disturbance (found

in the region surrounding Slave Lake). In the 2009 field season, this collection order was reversed such that scaup collection was completed near Slave Lake prior to the Athabasca region.

Location of collection of each bird was determined and documented using a hand-held GPS device (Garmin GPSMAP 76CSx, Olathe, Kansas, United States of America). Fresh body mass was recorded (to the nearest 5 g using a 1000g -Avinet Precision Spring Scale). Scaup carcasses were kept on ice after collection, and then frozen following return from the field each day.

2.2.2 *Sample Analysis and OCORT Quantification*

Scaup carcasses were thawed to allow dissection and removal of ovaries, which were then weighed (nearest 0.01 g on a scientific scale). Ovaries were examined for the presence of postovulatory follicles (POF), oviductal follicles (OF) and rapidly growing follicles (RGF) (Figure 2.2) (Johnson, 1990). All RGF and OF were removed from the ovary and weighed individually to the nearest hundredth of a gram. All RGFs greater than or weighing 0.5 grams, as well as OFs were used for CORT radioimmunoassay. The POFs were counted to determine the number of eggs that had been laid prior to collection. In 2008, few scaup had ovaries that were sufficiently developed for OCORT quantification (possibly due to timing of collection). Scaup with ovaries sufficient for analyses were collected later in the season, and were biased towards areas of oil and gas disturbance. Therefore only scaup ovaries from 2009 (n= 26) were used for OCORT analysis.

The presence of OCORT was evaluated using MP Biomedicals ImmuChemTM Double Antibody Corticosterone 125-I RIA Kits (Santa Ana, California, United States) (Sorenson et al., 1997). Standards were prepared in charcoal-stripped yolk (CSY). Distilled water (10 ml) was added to 10 g of randomly sampled scaup yolk, vortexed and subjected to a sonicator until a smooth consistency was obtained. The mixture was then added to another tube containing 0.4 g of charcoal, and rinsed with 10 ml of steroid diluent (ImmuChem Steroid DiluentTM supplied in the RIA Kit, Santa Ana, California, United States). The mixture was vortexed, allowed to sit overnight, and centrifuged the next day for 20 min. This process was repeated on the supernatant the next day, resulting in a 1:4 yolk to diluent ratio. A measured amount of CORT was then added to the CSY in order to obtain standards with CORT concentrations of 0, 0.125, 0.25, 0.5, 1, 2.5 and 5 ng/ml CSY. These standards were used to generate a standard curve from which OCORT concentrations of our samples were determined.

To prepare samples, follicles were cut in half and one half was placed into a large centrifuge tube with equal amount of distilled water (1 g=1 ml). The follicle and water mixture was homogenized, then diluted with steroid diluents such that a 1:4 (follicle:liquid) ratio was achieved. Standards and samples (0.5 ml) were extracted into 4 ml diethyl ether. The yolk mixture and ether were vortexed several times over one hour, placed in an ultracold freezer for 1/3 of an hour, and the ether supernatant poured into tubes and dried under air. The resulting precipitate was dissolved in 50 µl of ethanol and 450 µl of steroid diluent. Duplicate aliquots of these standards and samples were then analyzed according to kit instructions. Intra-assay coefficients of variation for the CORT radioimmunoassay for samples were 2.6% and 5.3% with means of 0.23 and 1.98 g/dl, respectively.

To estimate total ovarian CORT content, CORT content in RGFs and OFs were calculated separately and CORT content in POFs was estimated. The RGF CORT content was determined by multiplying the CORT concentration evaluated in each follicle by its respective total wet mass. The sum of CORT content across all RGFs determined RGF CORT in the ovary:

$$\text{RGF CORT (ng)} = \sum ([\text{RGF CORT}_i\,(\text{ng/g})] \times \text{RGF MASS}_i\,(\text{g}))$$

where i represents each RGF in the follicular hierarchy. The OF CORT concentration was determined by multiplying the CORT concentration evaluated in the OF by its respective total wet mass. If an OF was damaged/incomplete, OF CORT was estimated using an average OF mass (calculated from complete OFs obtained from collected scaup in 2008 and 2009):

$$\text{OF CORT (ng)} = [\bar{x}\ \text{OF CORT (ng/g)}] \times \text{OF MASS (g)}$$

The POF CORT concentration was estimated by multiplying the average OF mass (calculated from complete OFs obtained from collected scaup in 2008 and 2009) by the average CORT concentration obtained from RGFs and OF (if present) evaluated for that individual scaup.

$$\text{POF CORT (ng)} = ([\text{RGF \& OF CORT (ng/g)}] \times (N*20.32[\text{g}])$$

where N is the total number of POFs, and 20.32 (SD= 2.72) represents the average calculated mass (in grams) of OFs dissected from collected scaup. Total OCORT was then calculated by summing the total OCORT quantity across POF, OF, and RGFs;

OCORT (ng/g) = (RGF CORT + OF CORT + POF CORT)

2.2.3 *Nest Initiation Date Calculation:*

Assuming that scaup lay eggs at the rate of one per day (Batt et al., 1992), NID was estimated by counting the number of POFs, and counting the number of days backwards from date of collection. When POFs were not available, I generated a formula by regressing the mass of dissected OF and RGFs against the date of expected laying based on size in the follicular hierarchy (Figure 2.3, procedure derived from (Esler, 1994). For the 2008 field season I used the formula y = -0.30x + 1.36 9 (SE= 0.25), and for the 2009 field season I used the formula y = -0.30x + 1.39 (SE= 0.21) where x is the mass of the follicle, and y is the number of days projected from date of collection). As scaup typically lay eggs in the early morning (Austin et al., 2000), values of estimated NID were rounded to the day closest to morning hours and where possible and cross-referenced against recorded time of collection. For example, if a scaup was collected at 15:00 on June 2nd with an estimated NID in 2.5 days, I would estimate the NID to be June 5th.

2.2.4 *Habitat Quantification:*

Agricultural disturbance and natural landscape characteristics were obtained from the Enhanced Wetland Classification (EWC) data layer obtained from Ducks Unlimited, while industrial-type disturbances such as well pad disturbances and linear transportation data ([km] of rail and road disturbance) were obtained from HIS Incorporated. As both EWC and IHS map layers contained overlapping regions of data; these map layers were combined utilizing ArcView

10 to create one map layer so that no landscape features would be over-represented. A summary of all habitat attributes examined can be found in Table 2.1. Road and well disturbance were converted to a density measure (meters linear disturbance/m^2, or number of wells/buffer, respectively), while areas of agricultural disturbance and natural landscape characteristics were measured as a proportion of the buffer. These explanatory variables were converted to a z-score in order to normalize the data.

After creating a single map layer, habitat data within circular areas (buffers) of radii measuring 100 m, 250 m, 500 m, 1500 m, and 5000 m were extracted using ArcGIS 10 (Environmental Systems Research Institute [ESRI], 2010, Kranzberg, Deutschland) around the collection point for each scaup. The 500 m buffer represents the average observed home range of breeding scaup, while buffer radii of 250 m and 750m could represent the minimum and maximum observed home ranges observed of those pairs, respectively (Hammel, 1973). The 100 m buffer radius was selected to determine the effects of immediate habitat variables, while buffers of radii 1500 m and 5000 m were utilized to determine macro-effects of landscape on scaup.

2.2.5 *Statistical Analysis:*

Data were analysed using the free statistical computing software R (R Development Core Team, 2010). Prior to analysis, OCORT data were evaluated for normality by visual inspection of a histogram, followed by performing the Shapiro-Wilk test for normality which confirmed a non-normal distribution ($W=0.8853$, $p=0.00025$). Ovarian CORT was then log-transformed in

order to normalize the data prior to statistical analysis. All habitat variables and biological variables (body mass and OCORT) were converted to z-scores to normalize data.

For statistical analysis, I constructed *a priori* candidate models for each of the five buffer sizes across each of the response variables OCORT, BM, and NID (see Appendix 1). These models were selected and constructed based on the current knowledge of habitat characteristics favoured by breeding scaup (i.e., deep, stable permanent/ seasonal, and/or semi-permanent wetlands, lakes, and ponds (Austin et al., 1998; Corcoran et al., 2007; Fast et al., 2004)), in addition to potential perceived disturbances that may negatively impact scaup OCORT, BM, and NID (i.e., oil rigs (Barry et al., 1976)). Care was taken to develop similar models across buffer sizes for each dependent variable to ensure comparability in the final results, while allowing for the addition of new models due to landscape variation. Statistical null models (intercept only) and global models (including all explanatory variables) were included in each of the 15 model sets.

General linear modeling was applied to the candidate model sets. For each model an Akaike Information Criterion (AIC) value, and parameter estimates were generated for each explanatory variable using the add-on package MuMIn (Barton, 2010). Due to small sample size (n = 27 for OCORT analysis, and n = 47 for BM and NID analyses) in relation with the number of estimated parameters (10 variables), AIC values were converted to an AICc to correct for sample size (Anderson, 2008). To differentiate between models, I used model-averaging (Anderson, 2008) using the add-on package AICmodavg (Mazerolle, 2010) in R and calculated an 85% confidence interval (Arnold, 2010).

Post-hoc exploratory analyses were conducted to assess whether landscape characteristics operated on multi-scale levels to impact OCORT, BM, and NID. Habitat variables and other

variables that appeared to strongly correlate with OCORT, BM, and NID were combined (see Appendix 1) and compared to *a priori* candidate models at comparable buffer levels.

2.3 Results:

Totals of 85 female scaup (38 for (2008), and 47 for (2009)) were collected, of which 47 birds (n = 21 in 2008, n= 26 for 2009) had ovaries sufficiently developed enough to calculate an approximate nest-initiation date.

Analysis revealed that BM was comparable between years: 2008 ($\bar{x}$ = 795 g, SD= 67.63) similar to birds collected in 2009 ($\bar{x}$ = 815 g, SD= 42.73) (t [df= 32] = 1.17, p= 0.25). Average NID was June 9th in 2008 ($\bar{x}$ = 161, SD= 1.5) and June 10th in 2009 ($\bar{x}$ = 161, SD= 2.9), and not different from one another (t [df= 38] = 0.28, p = 0.78). Annual effects on BM were limited, as evidenced by graphs separating effects of habitat by year (see Appendix 2).

2.3.1 *Ovarian Corticosterone (OCORT)*

Best approximating models (ΔAICc < 2.0) explaining OCORT content for scaup can be found in Table 2.2, with model-averaged parameters presented in Table 2.5. In the 100 m, 250 m, 1500 m, and 5000 m buffers, scaup OCORT was best predicted by the DATE model as well as being a competing model in the 500 m buffer. In the 500 m buffer, OCORT was best predicted by the AGRIC+WATER; which also appeared in the 250 m buffer as a competing model. Other competing models included the null statistical model, BOGSFENS, SWAMP, TRANSPORT, and WATER. As a result of model selection uncertainty, model averaging was used to characterize the parameters most associated with OCORT. Model averaging identified SWAMP

to be negatively associated with OCORT at the 100 m buffer levels, while WATER was negatively associated with OCORT at the 1500 m buffer level (Table 2.5). Other parameters had little support as standard errors were large, and the 85% confidence intervals overlapped zero. As predicted, both SWAMP and WATER were negatively associated with OCORT (Table 2.2).

2.3.2 *Body Mass*

Best approximating models (ΔAICc < 2.0) explaining body mass for scaup can be found in Table 2.3, with model-averaged parameters presented in Table 2.6. In the 100 m, 250 m, and 500 m buffers; scaup body mass was best predicted by the null statistical model. Other competing models included AGRIC, BOGSFENS, OCORT, TRANSPORT, UPLAND, WATER, WELLS, WETLAND, and OCORT+TRANSPORT. At the 1500 m and 50000M buffer scaup body mass was best predicted by the WETLAND model, while competing models included the null statistical model, AGRIC, OCORT, SWAMP, TRANSPORT, OCORT+TRANSPORT. As a result of model selection uncertainty, model averaging was used to identify the variables most associated with body mass. Of the parameters tested, only WETLAND was found to be negatively associated with body mass (Table 2.3) at the 1500 m and 5000 m buffers. Other variables tested had little support as standard errors were large, and the 85% confidence intervals overlapped zero.

2.3.3 *Nest Initiation Date (NID):*

Best approximating models (ΔAICc < 2.0) explaining NID for scaup can be found in Table 2.4, with model-averaged parameters presented in Table 2.7. In the 100 m, 250 m, and 500

m buffers; scaup NID was best predicted by the WATER model. Competing models included BOGSFENS, WATER, AGRIC+WATER, OCORT+WATER, TRANSPORT+WATER, and WATER+WELLS. In the 1500 m buffer, scaup NID was best predicted by the global model, while TRANSPORT+WELLS was a competing model. For the 5000 m buffer, scaup NID was best predicted by the AGRIC+TRANSPORT model. Other competing models included the AGRIC, AGRIC+OCORT, AGRIC+WETLAND, and AGRIC+TRANSPORT+OCORT. As a result of model selection uncertainty, model averaging was used to identify the variables most associated with NID. Of the parameters tested, AGRIC was found to be positively associated with NID at the 100 m, and 5000 m buffer levels; BOGSFENS was found to be negatively associated with NID at the 100 m, 250 m, and 1500 m levels; TRANSPORT was found to be positively associated with NID at the 5000 m level; and WATER was found to be negatively associated with NID for all buffers except at the 5000 m level (Table 2.7). At the 1500 M buffer, ANTHRO, BOGSFENS, SWAMP, UPLAND, and AGRIC were negatively associated with NID while WELLS were positively associated (Table 2.7). Other parameters had little support as standard errors were large, and the 85% confidence intervals overlapped zero. As predicted, TRANSPORT were found to be positively associated with NID; while all sources of water (BOGSFENS, SWAMP, WETLAND, and WATER) were found to be negatively associated with NID (Table 2.1).

2.3.4 *Performance of Exploratory Models*

Best approximating models (ΔAICc < 2.0) explaining OCORT, BM, and NID for scaup at each observed buffer level can be found in Table 2.8, with model-averaged parameters

presented in Table 2.9. Exploratory models varied in their ability to predict OCORT, BM, and NID; but with the exception of one exploratory model at the 250 m predicting BM, they did not out-perform the *a priori* models previously tested. At the 100 m OCORT level, the best approximating models did not differ from the *a priori* models previously tested. Important model-averaged parameters did not change from the *a priori* analysis. At the 500 m scale, the model OCORT~ DATE + AGRIC(500) + WATER(500) was included in the best approximating models, with the *a priori* OCORT~AGRIC+WATER and OCORT~DATE models. Important model-averaged parameters included WATER which was present in the *a priori* analysis, and also included AGRIC as a strong negative predictor of OCORT. At the 250 m scale for BM, the exploratory model BM~ OCORT+TRANSPORT(250) +WETLAND(1500) was the best approximating model, outperforming all *a priori* models. With the exception of the BM~UPLAND model, all competing models were the same as in the *a priori* analysis. Model averaging yielded no significant predictors at this buffer level. At 1500 m buffer scales, exploratory models did not predict BM or NID better than the previously tested *a priori* models, although the NID~TRANSPORT+WELLS model was added as a competing mode for the 1500 m NID buffer. At the 1500 m BM level, significant parameters determined through model-averaging did not differ from the *a priori* models previously tested. However, at the 1500 m scale for NID, significant model-averaged parameters expanded to include AGRIC, ANTHRO, BOGSFENS, and WATER.

2.4 Discussion:

Overall, natural habitat and landscape features appear to contribute positively to scaup reproductive success. In the presence of water-based habitats (such as BOGSFENS, SWAMP, WETLAND, and WATER), OCORT was observed to be lower, NID were earlier, and scaup collected within these areas tended to have higher body mass. In contrast, birds collected in areas of high anthropogenic disturbance (ANTHRO, AGRIC, TRANSPORT, and WELLS) were typically delayed in NID, whereas impacts on OCORT and body mass appeared to be limited.

Unlike their mammalian counterparts, avian offspring grow and develop inside an egg. It is hypothesized that mothers deposit hormones into their eggs to ``communicate'' to their offspring regarding present environmental conditions (Groothuis et al., 2005). For example; female European starlings (*Sturnus vulgaris*) in better maternal body condition deposited less CORT into clutches than starlings in poor condition, possibly indicating ideal environmental conditions to their young (Love et al., 2008). In this study when SWAMP (100 m buffer) and WATER (500 m buffer) were more available to nesting females, OCORT was low, possibly indicating ideal conditions to future offspring. Scaup often choose large (>0.01 hectares), deeper, and stable permanent/ seasonal, and/or semi-permanent wetlands, lakes, and ponds (Austin et al., 1998; Corcoran et al., 2007; Fast et al., 2004) for nesting habitat. Female scaup in the Northwest Territories boreal forests were more likely to choose deeper and natural wetlands with amphipods, presumably as they provide a food resource for both ducklings and adults alike (Fast et al., 2004). Similarly, large water sources may provide protection against potential predators by providing a buffer preventing predators from accessing prey. Common eider (*Somateria mollissima*) ducklings displayed increased clustering behaviour in the water when faced with

surfing/boating disturbance in the water; however, when faced with shore-based activities (such as fishermen, cars, or pedestrian activity along the shoreline) they did not react as strongly (Keller, 1991). In these instances, water habitats may provide breeding scaup with security from potential stressors at a distance.

Scaup females were more likely to raise their broods on wetlands with the presence of yellow water lilies, possibly to conceal young ducklings from perceived predators (Fast et al., 2004). Thus, emergent vegetation in SWAMP and WATER habitats may provide scaup (and offspring) with a means to hide from predators, potentially lessening OCORT. Female scaup nesting in wooded creeks in boreal forests of the Yukon Flats of Alaska had higher nest success and duckling survival than females that raised their broods in wetland habitats (Corcoran et al., 2007). The combination of ideal foraging habitats for future offspring, as well multiple opportunities for escaping perceived predators may be why OCORT levels were lower in these habitats.

At the 1500 and 5000 m buffers, WETLAND was a negative predictor of body mass, suggesting that scaup utilizing landscapes with more wetlands weighed less than scaup in other habitats. While this may appear counterintuitive, evolutionary traps occur when individuals choose to breed in areas using environmental cues which may have signified high habitat quality in the past, but may concurrently then lead to failed nesting due to anthropogenic disturbances. It may be possible that WETLAND is not negatively impacting scaup body mass, but rather the impacts of anthropogenic disturbances within the vicinity of where scaup were collected. As we were only able to collect scaup within access of nearby roads, sample collection may be biased towards certain landscapes. Female scaup nesting in wooded creeks on the Yukon Flats in Alaska had higher nest success and duckling survival than females that had raised their broods in

wetland habitats (Corcoran et al., 2007). This difference may be due to landscape changes in the vicinity of the studied wetland habitats and may have acted to congregate predators as well as crowd nesting scaup females together (Corcoran et al., 2007). The occurrence of multiple breeding scaup in one location may increase competition for food (Corcoran et al., 2007), possibly leading to decreased body mass.

It may be possible that in an attempt to protect and ensure survival of the clutch, scaup may remain concealed on the nest at the cost of reducing foraging behaviour, resulting in decreased body mass. Nutrient reserve analysis of scaup collected in Erikson, Manitoba determined that (on average); incubating scaup were estimated to lose an average of 5.33g/day in initial body mass over an estimated 26-day incubation period. At the conclusion of incubation it was estimated that female scaup would experience a total 20% reduction in body mass, or roughly 139 g in body weight (Afton & Ankney, 1991). Scaup take short recesses during incubation, approximately 2.8 breaks per day averaging approximately 76 minutes in duration (Afton & Paulus, 1992), presumably to feed and replenish lipid stores. However, increased nest attendance has been positively related to nest success. Common eider females with high attendance had greatly increased nest survival; as much as 20% greater than females who had lower attendance (Andersson & Waldeck, 2006). Furthermore, incubation constancy was positively related to body condition for various waterfowl species studied (Afton & Paulus, 1992), suggesting that scaup nest attending behaviour may decrease with decreasing body mass. Scaup body mass may decrease with increased time spent on the nest, especially if scaup do not take recesses to replenish depleted nutrient reserves.

In this study, scaup initiated nests earlier with increased WATER habitat (250 m and 500 m buffers). It is hypothesized that scaup must first attain a certain amount of nutrient reserves

before rapid follicular growth (RFG) so that egg laying can occur (Esler et al., 2001; Gorman et al., 2008). Female scaup in RFG studied at the Yukon Flats National Wildlife Refuge, Alaska had on average 22.1 +/- 9.0 g (SE) in endogenous lipid, and 6.5 +/- 2.8 g (SE) protein more than scaup that had not initiated RFG (Esler et al., 2001). Similarly, non-reproductive greater scaup (*Aythya marila*) were noted to have lower lipid, protein, and mineral reserve levels compared to reproductively-active females (Gorman et al., 2008). It has been determined that lipid contribution into scaup clutches are of endogenous origin, whereas protein and mineral stores were from exogenous sources (Esler et al., 2001). Hence, scaup arriving on the breeding grounds with low body mass may require more foraging time in breeding areas before reaching the threshold required to develop eggs (Afton & Anderson, 2001). These females may be delayed in nesting compared to those birds arriving at the same time, but are heavier in mass and are able to initiate nests earlier (Esler et al., 2001). This also suggests that a female scaup arriving earlier to the breeding grounds, with similar body mass compared to a later- arriving female, may initiate her nest earlier if she is able to acquire additional reserves (Gorman et al., 2008). In a two-year study assessing nutrient-reserve dynamics in greater scaup, it was observed that higher water levels in one year resulted in more scaup reaching the nutrition threshold compared to the later year where water levels were lower (Gorman et al., 2008). This may also explain why NID is earlier with increased BOGSFENS, SWAMP, and WETLAND at the 1500 m buffer level as well. The presence of water-based habitats, specifically within the home-range of these breeding birds, may provide additional foraging areas to assist scaup in reaching the threshold required for reproduction.

In this study, when AGRIC increased, NID was prolonged at both the 100 m and 5000 m buffers. In other words, higher AGRIC disturbance was correlated with higher nesting in scaup.

With AGRIC development there is typically the removal of boreal forest that leads to increased presence of forest edges. The edge effect describes the phenomenon of the high diversity of plants and animals that occur at the edge of a habitat patch, or other landscape element (Forman, 1995). A higher occurrence of predator activity and disturbance have also been noted to occur at forest edges (Bayne & Hobson, 1997; Bayne & Hobson, 1998; Zegers, May, & Goodrich, 2000), possibly due to edges providing a higher selection of prey species, and providing predators an easily accessible travel route (see (Andren, 1995)for review). A higher density of predators may have negative consequences for waterfowl breeding close to landscape edges. In a meta-analysis of 64 experiments assessing the impact of edges on nest predation, natural nests as well as nests that were experimentally placed on the ground were significantly impacted by the presence of edges; while elevated nests above ground were less impacted (Batary & Baldi, 2004). The meta-analysis also revealed that edge effects significantly impacted nest predation in both forested and field edges (Batary & Baldi, 2004). Predators may negatively impact NID of breeding waterfowl. Presence of arctic foxes (*Alopex lagopus*) was sufficient to significantly alter NID of breeding dark-bellied Brent geese (*Branta bernicla bernicla*) from mid-June until early July; even causing some geese to abandon attempts at breeding altogether (Spaans et al., 1998). Other studies found no significant effect of edges on waterfowl nesting success. Nest success of mallards, gadwall (*Anas strepera*), and blue-winged teal (*Anas discors*) studied in the prairie pothole region of Saskatchewan was found to be unrelated to proximity of wetland, road, or habitat edges (Pasitschniak-Arts, Clark, & Messier, 1998). In contrast, upland-nesting ducks (including scaup) found nesting within 400M of a field edge in the high-grassland areas of North Dakota were found to have lower nest success (38%) compared to ducks who had nested >400M away from field edges, which displayed higher nesting success (71%) (Horn et al., 2005). However,

variation in edge effect in the prairie pothole regions could be caused by multiple factors. Predator composition and diversity for example, could determine whether edge effects are observed depending on the predator's search pattern, and whether or not predators utilize edges when searching for prey species (Andren, 1995; Marini, Robinson, & Heske, 1995). The prairie pothole region specifically has seen a transition in predator community since human development began in the late 1800s (Sargeant et al., 1993), possibly resulting in an abundance of "generalist" predators (Angelstam, 1986). Generalist predators are flexible in their ability to move between habitats during their daily activities, and furthermore may not have a strong preference for certain habitats, unlike specialist predators (Andren, 1995). Grazing livestock and close proximity of human activities/disturbances (Blas et al., 2007) may disrupt nesting resulting in delays. Alternatively, general trends suggest that predators are more likely to respond positively (in terms of abundance, species richness, or activity) to fragmentation occurring at landscape vs. smaller scales (such as edge of patch spatial scales) (Stephens et al., 2003). Interestingly, it is at intermediate landscape scales that nesting success of waterfowl is hampered. Upland-nesting ducks studied in the prairie-pothole regions of North Dakota demonstrated a curvilinear relationship with high nest success in both small and large fields, but low nest success in moderate-sized fields (Horn et al., 2005). The proposed hypothesis was that mammalian predators may overlook small patches of habitat, and as a result nest success in small habitat patches will be high yet variable (Horn et al., 2005). Conversely, predators may be ineffective at searching for nests in larger fields, or may be unsuccessful in nest searches due to different predator composition in larger landscapes as opposed to smaller spatial scales (Horn et al., 2005). Habitats of intermediate size offer a viable alternative to predators. Striped skunks (*Mephittis mephitiis*) and red foxes (*Vulpes fulva*) in North Dakota spent a disproportionate

amount of time in intermediate-sized fields (Phillips et al., 2004). Discrepancies in the results between the above study (Horn et al., 2005) and my own may have been due to the different habitat classification methods, as well as the surrounding landscape attributes. Proportion of UPLAND habitat for each scaup collected in my intermediate buffer sizes in this study varied (ranging from 0% to almost 90% coverage), whereas the study areas assessed by Horn et al 2005 consisted almost entirely of grassland and UPLAND habitats (>74% for all habitats assessed). Patch sizes assessed also differed; Horn et al (2005) assessed fields ranging from 2-606 ha (or a buffer radius ranging from 80m to 1389m, respectively). It is possible that boreal forest habitats of Alberta are so fragmented (Norton, Hannon, & Schmiegelow, 2000; Schmiegelow & Monkkonen, 2002) that predators stand the best chance of finding prey at smaller spatial scales. This predator pressure may result in delayed NID for breeding scaup. In contrast, it may be that at the 5000 m buffer zone, the cumulative effects of increased forest edges, disturbances, and potentially the addition of new predators with larger home-ranges, may negatively impact scaup NID at the landscape level. The largest field assessed by Horn had an area of 606 ha, or a buffer radius of approximately 1390m, making it difficult to determine whether the curvilinear relationship observed could be extrapolated to our buffer size of 5000 m. Predator activity may thus be dispersed throughout the landscape and not concentrated at edges, diffusing potential edge effects.

An increasing density of roads and railway tracks (TRANSPORT) was associated with an increase in NID at the 5000 m buffer level. Scaup breeding in the boreal forests of the Northwest Territories preferred natural wetlands that were large and deep (Fast et al., 2004), with amphipod abundance and area of wetland as important predictors as to whether or not a wetland was utilized by a breeding female (Fast et al., 2004). However, female scaup often choose to nest on

borrow pits (depressions made by excavation of material during road construction) as they provide an ideal habitat for certain amphipod species (Bartonek & Murdy, 1970), and may supply optimal feeding sites for scaup for short periods of time (Bartonek & Murdy, 1970; Lindeman & Clark, 1999). While prey abundance may be comparable between natural ponds and borrow pits (with amphipods found in 92% and 67% of ponds respectively), there are other factors which may negatively impact scaup reproductive success. Natural ponds in the boreal forests of the Northwest Territories supported a median density of 2.5 broods/ha (0.0-8.6), while borrow pits supported 1.7 broods (0.0-50.0) (Walsh et al., 2006). While scaup pairs were frequently seen on borrow pits, females raised their broods almost exclusively on natural wetlands, potentially due to the higher survival as a result of better food resources and emergent vegetation which may act to shield ducklings from potential predators (Fast et al., 2004). The discrepancy in brood usage might also be a result of the physical and chemical differences between natural ponds and borrow pits, as natural ponds are significantly larger and deeper than borrow pits (Walsh et al., 2006), while borrow pits have higher pH, conductivity, and total dissolved solids than natural ponds (Walsh et al., 2006). Females may be drawn to borrow pits because of food resources, but transport disturbances may delay NID.

Waterfowl species are highly sensitive to disturbance during the breeding season, and respond both to rapid movements and loud noises (Korschgen & Dahlgren, 1992). Diving ducks have been reported to be especially vulnerable to the road disturbance (Korschgen & Dahlgren, 1992). Pink-footed geese (*Anser brachyrhynchus)* were disturbed by traffic as far as 500 m away, while populations of Eurasian coots (*Fulica atra*) and Northern shovelers (*Anas clypeata*) were suppressed of up to a distance 20m at low traffic volume levels (Reijnen, Foppen, & Meeuwsen, 1996). Increasing the amount of traffic may also increase the effective disturbance area. At a

traffic load of 50 000 cars/day, populations of Eurasian coots and Northern shovelers were further suppressed up to a distance of 75m and 320m respectively from the source of traffic (Reijnen et al., 1996). Not surprisingly, TRANSPORT disturbances may also decrease habitat use by certain species in areas surrounding roads. Pink-footed geese decreased the use of habitats in the neighbouring areas with traffic rates as little as 10 cars per day, (Korschgen & Dahlgren, 1992). Populations of coots and Northern shovelers were estimated to decrease approximately 10% and 35% respectively when within 0-100 m of a road with a traffic load of 5000 cars/day travelling at approximately 120 km/hour (Reijnen et al., 1996). TRANSPORT disturbances can cause waterbirds to flush and/or reduce foraging behaviour, depleting valuable energy stores required during the critical nest-initiation period. Wading birds studied at a wildlife reserve in Florida flushed when exposed to an approaching vehicle (Stolen, 2003), while snowy egrets (*Egretta thula*) and great egrets (*Ardea alba*) subjected to vehicle disturbances significantly decreased foraging rates (Stolen, 2003). Migrant ducks arriving in Florida (including blue-winged teal (*A. discors*), mottled duck (*A. fulvigula*), Northern pintail (*A. acuta*), American coot (*Fulica americana*), American wigeon (*A. americana*), and Northern shoveler avoided foraging areas within the vicinity of roads within the first few months of arriving on the feeding grounds (Klein, Humphrey, & Percival, 1995). Scaup arriving in the boreal forests may be negatively impacted by TRANSPORT disturbances, and may possibly alter foraging regimes and nest site selection behaviours, resulting in delayed NID.

An increase in the proportion of oil pad wells (WELLS) in the landscape was associated with a prolonged NID at the 1500 m buffer level. Anecdotal evidence dating as far back from 1904 noted that waterfowl diversity had been severely depressed in the vicinity of oil derricks located in the Grand Reservoir in Ohio, and that young ducklings had not been observed for four

years despite mallards and blue-winged teal being known to nest in the area (Henninger, 1904). Species including cormorants (*Phalacrocorax* species) and gadwall had not been observed to nest in the surrounding area for several years (Henninger, 1904). Sage grouse (*Centrocercus urophasianus*) within 3km of roads and well pads had a nest initiation of only 65%, while grouse from leks that were >3 km away from disturbances, or separated by topographic features had nest initiation rates of 89% (Lyon & Anderson, 2003). Several waterfowl species avoid well pads and compressor station sites from distances as low as 800m up to a distance of 8 km (Barry et al., 1976; Bromley, 1985). While there is some evidence that particular waterfowl species may eventually become accustomed to the oil-wells and compression stations, observations have been limited to a few pairs which still maintain a wary distance from these new structures (Barry et al., 1976). Lesser scaup, specifically, had a strong preference for low-disturbance habitats, rather than sites in the vicinity of oil rigs (Barry et al., 1976). Scaup that nest in the vicinity of natural resource extraction activity, may experience delayed NID as a result of neighbouring disturbance , as occurred in sage grouse (Lyon & Anderson., 2003).

Further research is needed to understand the role landscape features play in waterfowl reproduction. A meta-analysis of journal articles from 1984 to 2000 found limited studies investigating the impacts of habitat characteristics on waterfowl reproduction (Stephens et al., 2003). Of the 86 publications reviewed, only 7.1% of studies assessed landscape impacts on duck nesting success, vs. the 76.2% of studies assessing landscape impact on *passeriformes* species (Stephens et al., 2003). As these studies were often spread out over time, multiple factors including land use changes, predator biodiversity, weather conditions make drawing conclusions very difficult (Clark & Nudds, 1991). Gaps in our understanding of waterfowl species and specific geographical regions, makes it difficult to definitively conclude the true impact of

landscape variables on waterfowl reproductive success (Stephens et al., 2003). At the present time, this project appears to be one of the few studies that attempts to address the issue of how landscape and anthropogenic disturbances can influence scaup reproduction on multiple spatial scales. The results of my study support that both natural features and human disturbances play important roles in various aspects that are required for successful scaup reproduction. Furthermore, my study highlights the need for a holistic approach to conservation management, considering not only local habitat characteristics and disturbances in the immediate vicinity of breeding waterfowl, but extending beyond and incorporating regional landscape attributes.

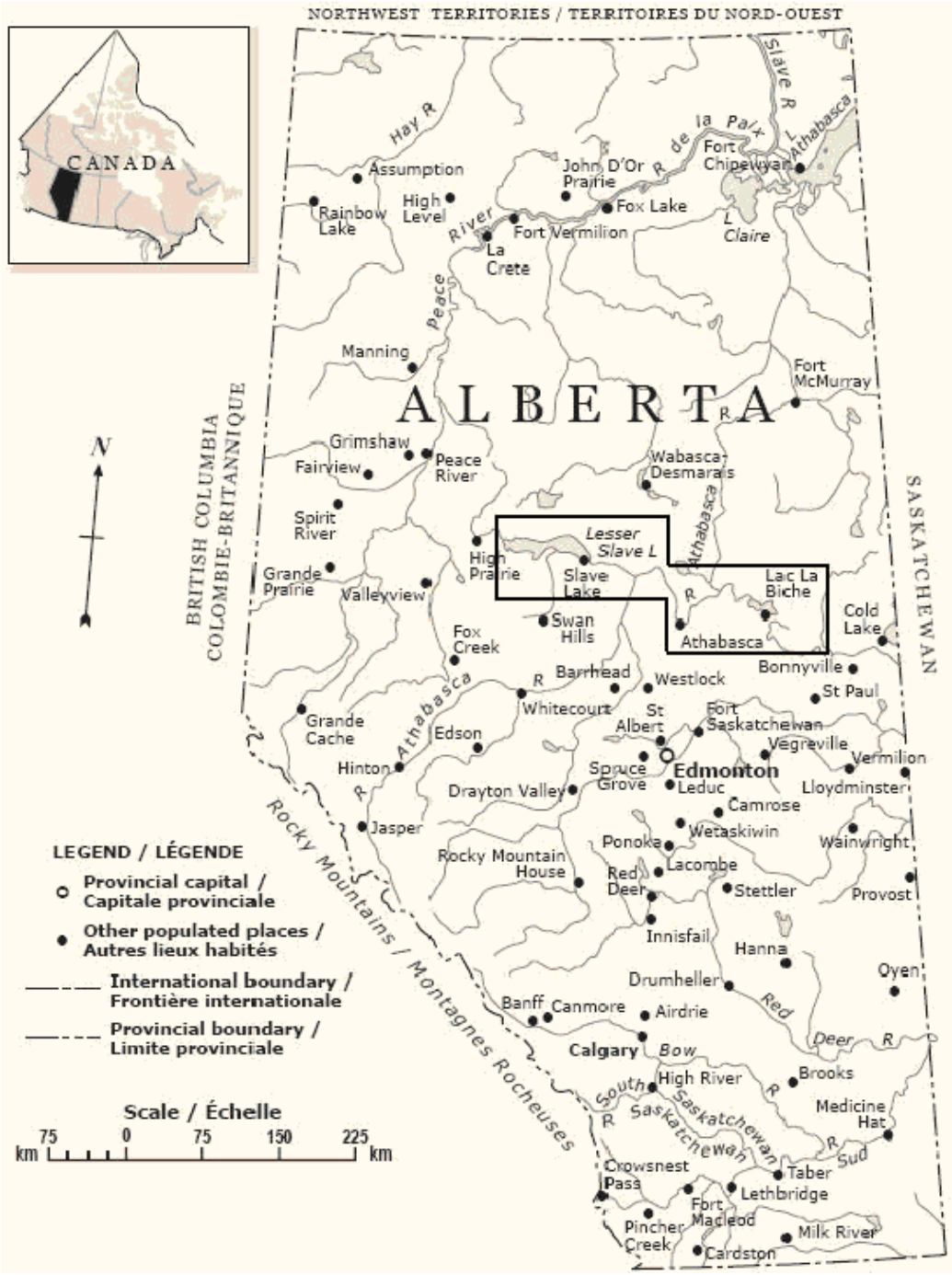

Figure 2.1: Map of Northern Alberta depicting area of scaup sample collection (black outline) in the field collection of June 2008 and 2009 (maps obtained and modified from Natural Resources Canada, http://atlas.gc.ca).

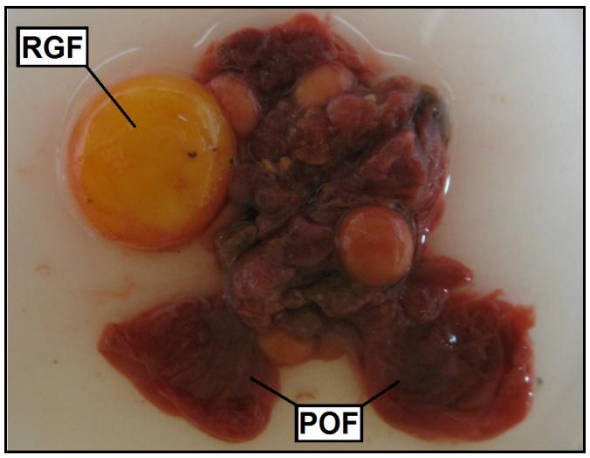

Figure 2.2: A dissected lesser scaup ovary displaying the stages of follicular development; rapidly-growing follicles (RGF) are attached to the ovary and are 0.5g in mass or heavier, and postovulatory follicles (POF) are the remnants of RGFs.

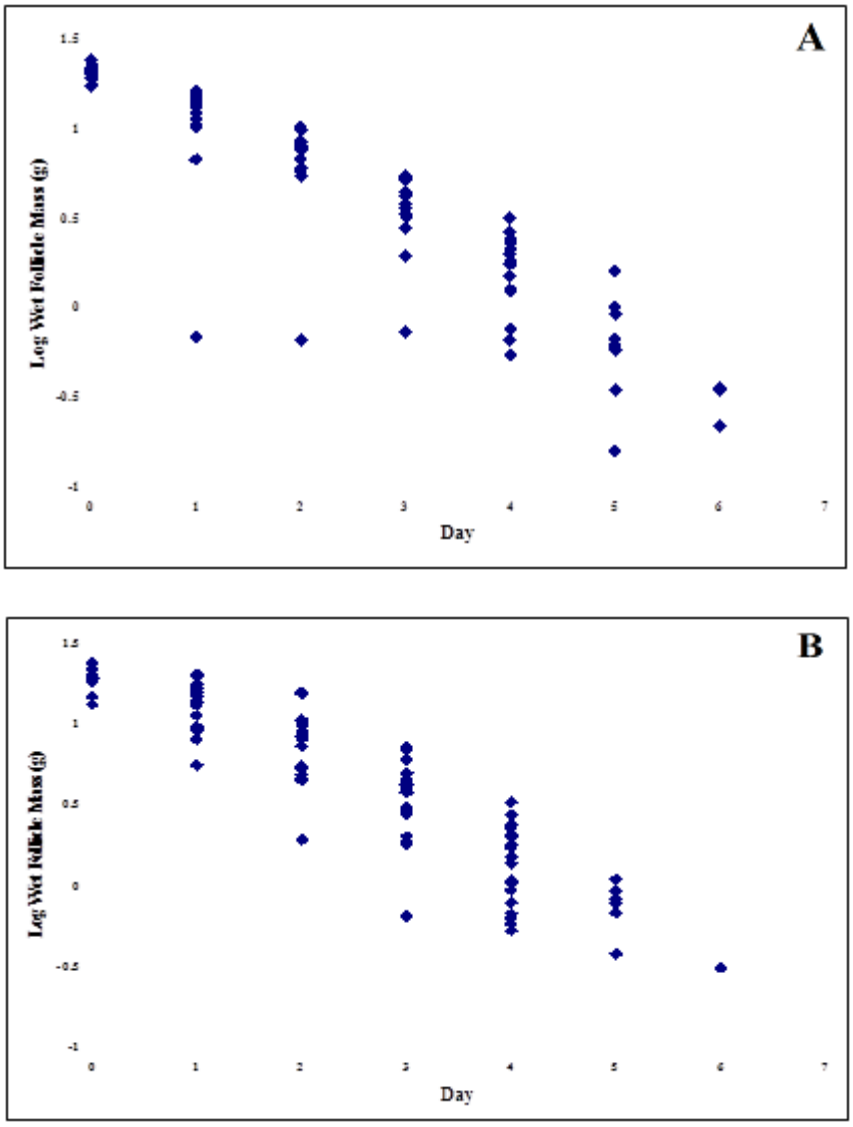

Figure 2.3: Graphs depicting the regression relationship between mass of lesser scaup oviductal and/or rapidly growing follicles across the date of expected lay (assuming one egg per day) for the (A) 2008 field season with n =15 scaup, and (B) the 2009 field season with n = 20 scaup. Using this graph we were able to derive an equation to estimate female lesser scaup nest initiation date when post-oviductal follicles were not available (Esler, 1994).

Table 2.1: Description of explanatory variables used in candidate set models for follicular CORT, NID, and BM for female scaup collected in Northern Alberta, Canada, in the summers of 2008/2009. Predicted results are represented as $\beta > 0$ if a positive correlation is predicted, and $\beta < 0$ if a negative correlation is predicted.

HABITAT	DESCRIPTION	EXPECTED RESPONSE (CORT, BM, NID)
BOGSFENS	Normalized proportion data including classes associated with peat wetlands; such as poor and rich fen classes (treed, shrubby, graminoid fens) as well as bog classes (treed, shrubby, and open bog) habitat.	$\beta < 0$, $\beta > 0$, $\beta < 0$
SWAMP	Normalized proportion data including mineral wetland swamp classes (tamarack and conifer swamp).	$\beta < 0$, $\beta > 0$, $\beta < 0$
UPLAND	Normalized proportion data including natural, undisturbed upland classes (conifer, deciduous, and pine mixed forest upland).	$\beta < 0$, $\beta < 0$, $\beta < 0$
WETLAND	Normalized proportion data including mineral wetland open water classes (aquatic bed and open water)	$\beta < 0$, $\beta > 0$, $\beta < 0$
WATER	Normalized proportion data including all water body sources combined (sum of BOGSFENS, SWAMP, and WETLAND).	$\beta < 0$, $\beta > 0$, $\beta < 0$
ANTHRO	Normalized proportion data including city dwellings (residential housing, buildings, etc.) and transportation disturbance.	$\beta > 0$, $\beta < 0$, $\beta > 0$
WELLS	Normalized point-count density of oil-pad wells.	$\beta > 0$, $\beta < 0$, $\beta > 0$
TRANSPORT	Normalized linear transportation density (road and railway tracks).	$\beta > 0$, $\beta < 0$, $\beta > 0$
OTHER		
OCORT	Follicular CORT content of collected female Lesser Scaup ovaries.	NA , $\beta < 0$, NA
DATE	Converted Julian Date of female Lesser Scaup scaup collection	$\beta > 0$, NA, NA
BM	Body Mass of collected female Lesser Scaup.	NA, NA, $\beta < 0$

Table 2.2: Best approximating models (ΔAICc < 2.0) explaining OCORT for scaup (n=26) collected in Northern Alberta in 2009 across the selected five buffer sizes. The table includes model description, the number of parameters (K), model deviance (DEV), the AICc difference between the highest ranked model for each buffer (ΔAICc), and model weight (*W*i).

Model Formula	K	DEV	AICc Value	ΔAICc	*Wi*
100 m					
OCORT~DATE	3	1.71	10.19	0.00	0.14
OCORT~SWAMP	3	1.73	10.47	0.28	0.13
OCORT~1	2	1.95	10.91	0.72	0.10
OCORT~WATER	3	1.81	11.59	1.40	0.07
250 m					
OCORT~DATE	3	1.71	10.19	0.00	0.15
OCORT~WATER	3	1.76	10.87	0.69	0.11
OCORT~1	2	1.95	10.91	0.72	0.11
OCORT~BOGSFENS	3	1.79	11.30	1.12	0.09
OCORT~SWAMP	3	1.81	11.60	1.41	0.08
OCORT~AGRIC+WATER	4	1.66	12.11	1.92	0.06
500 m					
OCORT~AGRIC+WATER	4	1.44	8.43	0.00	0.22
OCORT~DATE	3	1.71	10.19	1.76	0.09
1500 m					
OCORT~DATE	3	1.71	10.19	0.00	0.16
OCORT~1	2	1.95	10.91	0.72	0.11
OCORT~BOGSFENS	3	1.79	11.24	1.05	0.10
OCORT~WATER	3	1.79	11.26	1.07	0.09
5000 m					
OCORT~DATE	3	1.71	10.19	0.00	0.17
OCORT~1	2	1.95	10.91	0.72	0.12
OCORT~WATER	3	1.78	11.20	1.01	0.10
OCORT~TRANSPORT	3	1.80	11.41	1.23	0.09
OCORT~BOGSFENS	3	1.83	11.83	1.64	0.07

Table 2.3: Best approximating models (ΔAICc < 2.0) explaining body mass for scaup (n=47) collected in Northern Alberta in 2008 and 2009 across the selected five buffer sizes. The table includes model description, the number of parameters (K), model deviance (DEV), the AICc difference between the highest ranked model for each buffer (ΔAICc), and model weight (*W*i).

Model Formula	K	DEV	AICc Value	ΔAICc	*Wi*
100 m					
BM~1	2	119992.6	506.37	0.00	0.14
BM~OCORT	3	116158.1	507.13	0.76	0.10
BM~WETLAND	3	118603.1	508.11	1.74	0.06
BM~AGRIC	3	118701.8	508.15	1.78	0.06
BM~TRANSPORT	3	118969.3	508.25	1.88	0.06
250 m					
BM~1	2	119992.6	506.37	0.00	0.11
BM~OCORT	3	116158.1	507.13	0.76	0.08
BM~TRANSPORT	3	116393.0	507.22	0.85	0.07
BM~OCORT+TRANSPORT	4	111932.0	507.78	1.41	0.06
BM~AGRIC	3	118370.6	508.02	1.65	0.05
BM~WETLAND	3	118817.1	508.19	1.82	0.05
BM~WELLS	3	118920.8	508.23	1.86	0.04
BM~WATER	3	119078.3	508.30	1.93	0.04
BM~BOGSFENS	3	119189.6	508.34	1.97	0.04
BM~UPLAND	3	119211.3	508.35	1.98	0.04
500 m					
BM~1	2	119992.6	506.37	0.00	0.10
BM~TRANSPORT	3	116133.0	507.12	0.75	0.07
BM~OCORT	3	116158.1	507.13	0.76	0.07
BM~WELLS	3	117156.2	507.53	1.16	0.05
BM~WELLS+OCORT	4	112251.9	507.92	1.55	0.04
BM~WETLAND	3	118265.4	507.97	1.60	0.04
BM~OCORT+TRANSPORT	4	112395.3	507.98	1.61	0.04
BM~AGRIC	3	118408.4	508.03	1.66	0.04
BM~ANTHRO	3	118511.1	508.07	1.70	0.04
BM~BOGSFENS	3	118544.4	508.08	1.71	0.04
BM~SWAMP	3	118583.7	508.10	1.73	0.04
BM~WATER	3	119255.7	508.37	2.00	0.04
1500 m					
BM~WETLAND	3	108773.8	504.04	0.00	0.22
5000 m					

BM~WETLAND	3	112930.2	505.80	0.00	0.13
BM~1	2	119992.6	506.37	0.57	0.10
BM~TRANSPORT	3	115504.4	506.86	1.06	0.08
BM~SWAMP	3	115711.6	506.95	1.14	0.08
BM~OCORT	3	116158.1	507.13	1.32	0.07
BM~AGRIC	3	116932.9	507.44	1.64	0.06
BM~OCORT+TRANSPORT	4	111354.1	507.54	1.73	0.06

Table 2.4: Best approximating models (ΔAICc < 2.0) explaining NID (nest initiation date) for scaup (n=47) collected in Northern Alberta in 2008 and 2009 across the selected five buffer sizes. The table includes model description, the number of parameters (K), model deviance (DEV), the AICc difference between the highest ranked model for each buffer (ΔAICc), and model weight (*W*i).

Model Formula	K	DEV	AICc Value	ΔAICc	*W*i
100 m					
NID~AGRIC	3	212.84	207.57	0.00	0.13
NID~AGRIC+TRANSPORT	4	203.18	207.83	0.26	0.12
NID~AGRIC+TRANSPORT+OCORT	5	193.80	208.16	0.59	0.10
NID~AGRIC+OCORT	4	204.85	208.20	0.63	0.10
NID~BOGSFENS	3	217.22	208.50	0.94	0.08
NID~AGRIC+WETLAND	4	206.43	208.56	0.99	0.08
NID~AGRIC+ANTHRO	4	208.48	209.01	1.44	0.06
250 m					
NID~WATER	3	217.62	211.97	0.00	0.17
NID~TRANSPORT+WATER	4	208.24	212.29	0.32	0.15
NID~OCORT+WATER	4	210.48	212.80	0.83	0.11
NID~BOGSFENS	3	222.71	213.06	1.09	0.10
NID~WATER+WELLS	4	213.47	213.46	1.49	0.08
500 m					
NID~WATER	3	214.55	211.30	0.00	0.19
NID~BOGSFENS	3	218.74	212.21	0.91	0.12
NID~OCORT+WATER	4	210.13	212.72	1.42	0.10
NID~TRANSPORT+WATER	4	210.57	212.82	1.52	0.09
1500 m					
NID~AGRIC+ANTHRO+BOGSFENS+OCORT+BM+SWAMP+UPLAND+WETLAND+WELLS	11	116.60	205.63	0.00	0.43
5000 m					
NID~AGRIC+TRANSPORT	4	202.23	210.92	0.00	0.17
NID~AGRIC+TRANSPORT+OCORT	5	193.75	211.42	0.50	0.13
NID~AGRIC	3	216.70	211.77	0.85	0.11
NID~AGRIC+WETLAND	4	207.99	212.24	1.32	0.09
NID~AGRIC+OCORT	4	209.10	212.49	1.57	0.08

Table 2.5: Model-averaged parameter estimates across all models for significant indicators of predicted OCORT for Lesser Scaup ovaries collected from Northern Alberta in the summer of 2009 (n=26). Unconditional standard error (SE) and 85% confidence provided for each parameter.

Parameter	Model-Averaged Estimate	Unconditional SE	85% Confidence Interval Lower	Upper
100 m				
SWAMP	-0.09	0.05	-0.17	-0.01
500 m				
WATER	-0.18	0.12	-0.35	-0.01

Table 2.6: Model-averaged parameter estimates across all models for significant indicators explaining predicted body mass for Lesser Scaup ovaries collected from Northern Alberta in the summers of 2008 and 2009 (n=47). Unconditional standard error (SE) and 85% confidence provided for each parameter.

Parameter	Model-Averaged Estimate	Unconditional SE	85% Confidence Interval Lower	Upper
1500 m				
WETLAND	-15.63	10.24	-30.38	-0.89
5000 m				
WETLAND	-11.91	7.58	-22.83	-1.00

Table 2.7: Model-averaged parameter estimates across all models for significant indicators explaining predicted NID (nest-initiation date) for Lesser Scaup ovaries collected from northern Alberta in the summers of 2008 and 2009 (n=47). Unconditional standard error (SE) and 85% confidence provided for each parameter.

Parameter	**Model-Averaged**	**Unconditional**	**85% Confidence Interval**	
	Estimate	**SE**	**Lower**	**Upper**
100 m				
AGRIC	1.01	0.39	0.45	1.57
BOGSFENS	-0.89	0.42	-1.49	-0.3
250 m				
BOGSFENS	-0.81	0.33	-1.28	-0.34
WATER	-0.97	0.38	-1.51	-0.42
500 m				
WATER	-0.98	0.40	-1.56	-0.40
1500 m				
SWAMP	-7.10	4.88	-14.13	-0.07
TRANSPORT	-0.58	0.37	-1.12	-0.04
UPLAND	-13.96	8.50	-26.20	-1.72
WELLS	-1.13	0.37	-1.67	-0.60
WETLAND	-6.96	4.45	-13.37	-0.55
5000 m				
AGRIC	1.19	0.52	0.44	1.93
TRANSPORT	0.79	0.47	0.12	1.47

Table 2.8: Best approximating models (ΔAICc < 2.0) in the exploratory model set explaining OCORT, BM, and NID for scaup collected in Northern Alberta in 2008 and 2009. Only 2009 birds (n = 26) were included in the OCORT analysis, while birds collected from 2008 and 2009 (including accompanying calculated OCORT values) were used in BM and NID analyses (n=47). The table includes model description, the number of parameters (K), model deviance (DEV), the AICc difference between the highest ranked model for each buffer (ΔAICc), and model weight (*W*i).

Model Formula	K	DEV	AICc Value	ΔAICc	*W*i
100 m OCORT					
OCORT~ DATE	3	1.71	10.19	0.00	0.14
OCORT~ SWAMP	3	1.80	10.47	0.28	0.12
OCORT~ 1	3	1.95	10.91	0.72	0.10
OCORT~ WATER	3	1.73	11.59	1.40	0.07
500 m OCORT					
OCORT~ AGRIC + WATER	4	1.82	8.43	0.00	0.18
OCORT~ DATE + AGRIC(500 m) + WATER (500 m)	5	1.29	8.63	0.20	0.16
OCORT~ DATE	3	1.71	10.19	1.77	0.07
250 m BM					
BM~OCORT+TRANSPORT(250 m)+WETLAND(1500 m)	5	102898.2	506.34	0.00	0.10
BM~1	2	119992.6	506.37	0.03	0.10
BM~OCORT	3	116158.1	507.13	0.79	0.07
BM~TRANSPORT	3	116393.0	507.22	0.88	0.07
BM~OCORT+TRANSPORT	4	111932.0	507.78	1.44	0.05
BM~AGRIC	3	118370.6	508.02	1.68	0.04
BM~WETLAND	3	118817.1	508.19	1.85	0.04
BM~WELLS	3	118920.8	508.23	1.89	0.04
BM~WATER	3	119078.3	508.30	1.96	0.04
BM~BOGSFENS	3	119189.6	508.34	2.00	0.04
1500 m BM					
BM~ WETLAND	3	108773.8	504.04	0.00	0.20
1500 m NID					

NID~AGRIC+ANTHRO+BOGSFENS+ OCORT+BM+SWAMP+UPLAND+ WETLAND+WELLS	11	116.60	205.63	0.00	0.43
NID~TRANSPORT+WELLS	4	187.75	207.43	1.80	0.17

Table 2.9: Model-averaged parameter estimates for each buffer level including exploratory models for significant indicators explaining predicted OCORT, BM, and NID (nest-initiation date) for Lesser Scaup collected from Northern Alberta in the summers of 2008 and 2009. Only 2009 birds were included in the OCORT analysis (n=26), while birds collected from 2008 and 2009 (including accompanying calculated OCORT values) were used in BM and NID analyses (n=47). Unconditional standard error (SE) and 85% confidence provided for each parameter.

Parameter	Model-Averaged Estimate	Unconditional SE	85% Confidence Interval Lower	Upper
100 m OCORT				
SWAMP	-0.09	0.05	-0.17	-0.01
500 m OCORT				
AGRIC	-0.19	0.12	-0.37	-0.02
WATER	-0.19	0.1	-0.35	-0.03
1500 m BM				
WETLAND	-15.47	9.82	-29.60	-1.33
1500 m NID				
AGRIC	-35.42	23.01	-68.55	-2.29
ANTRHO	-11.48	5.72	-19.73	-3.24
BOGSFENS	-27.40	18.46	-53.98	-0.82
SWAMP	-8.73	4.01	-14.50	-2.96
UPLAND	-17.61	5.63	-25.71	-9.52
WATER	-1.01	0.34	-1.50	-0.52
WELLS	0.16	0.08	0.05	0.28
WETLAND	-8.43	3.64	-13.67	-3.18

2.5 References

Afton, A. D. (1984). Influence of age and time on reproductive performance of female lesser scaup. *The Auk, 101*, 255-265.

Afton, A. D., & Anderson, M. G. (2001). Declining scaup populations: A retrospective analysis of long-term population and harvest survey data. *Journal of Wildlife Management, 65*(4), 781-796.

Afton, A. D., & Ankney, C. D. (1991). Nutrient-reserve dynamics of breeding lesser scaup: A test of competing hypotheses. *Condor, 93*, 89-97.

Afton, A. D., & Paulus, S. L. (1992). Incubation and brood care. In Batt, B. D. J., Afton, A. D., Anderson, A. G., Ankney, C. D., Johnson, D. H., Kadlec, J. A., et al. (1992). *Ecology and management of breeding waterfowl*. United States of America: University of Minnesota Press.

Anderson, D. R. (Ed.). (2008). *Model based inference in the life sciences: A primer on evidence*. New York: Springer Science+Business Media, LLC.

Andersson, M. A., & Waldeck, P. (2006). Reproductive tactics under severe egg predation: An eider's dilemma. *Behavioural Ecology, 148*, 350-355.

Andren, H. (1995). Effects of landscape composiiton on predation rates at habitat edges. In L. Hansson, L. Fahrig & G. Merriam (Eds.), *Mosaic landscapes and ecological processes* (pp. 225-255). London, UK: Chapman & Hall.

Angelstam, P. (1986). Predation on ground-nesting birds' nests in relation to predator densities and habitat edge. *OIKOS, 47*, 365-373.

Arnold, T. W. (2010). Uninformative parameters and model selection using akaike's information criterion. *Journal of Wildlife Management, 74*, 1175-1178.

Austin, J. E., Afton, A. D., Anderson, M. G., Clark, R. G., Custer, C. M., Lawrence, J. S., et al. (2000). Declining scaup populations: Issues, hypotheses, and research needs. *Wildlife Society Bulletin, 28*(1), 254-263.

Austin, J. E., Anteau, M. J., Barclay, J. S., Boomer, G. S., Rohwer, F. C., & Slattery, S. M. (2006). Declining scaup populations: Reassessment of the issues, hypotheses, and research directions. *Second Scaup Workshop*, Mismarck, ND.

Austin, J. E., Custer, C. J., & Afton, A. D. (1998). Lesser Scaup(*Aythya affinis*). *The Birds of North America*, (338), 32.Barry, T. W., & Spencer, R. (1976). *Wildlife response to oil well drilling*. Ottawa, ON: Canadian Wildlife Service.

Barry, T. W., Spencer, R., & Canadian Wildlife Service. (1976). *Wildlife response to oil well drilling* (Progress Notes. Ottawa: Canadian Wildlife Service.

Barton, K. (2010). *MuMIn: Multi-model inference* in CRAN. Developed for R Development Core Team, . (2010). *R: A language and environment for statistical computing*. Vienna, Austria:

Bartonek, J. C., & Murdy, H. W. (1970). Summer foods of lesser scaup in the subarctic taiga. *Arctic, 23*(1), 35-44.

Batary, P., & Baldi, A. (2004). Evidence of an edge effect on avian nest success. *Conservation Biology, 18*(2), 389-400.

Batt, B. D. J., Afton, A. D., Anderson, A. G., Ankney, C. D., Johnson, D. H., Kadlec, J. A., et al. (1992). *Ecology and management of breeding waterfowl*. United States of America: University of Minnesota Press.

Bayne, E. M., & Hobson, K. A. (1998). The effects of habitat fragmentation by forestry and agriculture on the abundance of small mammals in the southern boreal mixedwood forest. *Canadian Journal of Zoology, 76*, 62-69.

Bayne, E. M., & Hobson, K. A. (1997). Comparing the effects of landscape fragmentation by forestry and agriculture on predation of artificial nests. *Conservation Biology, 11*(6), 1418-1429.

Blas, J., Bortolotti, G. R., Tella, J. L., Baos, R., & Marchant, T. A. (2007). Stress response during development predicts fitness in a wild, long lived vertebrate. *Proceedings of the Royal Society of London, 104*(21), 8880-8884.

Blums, P., Mednis, A., & Clark, R. G. (1997). Effect of incubation body mass on reproductive success and survival of two european diving ducks: A test of the nutrient limitation hypothesis. *The Condor, 99*(4), 916-925.

Blums, P., Nichols, J. D., Hines, J. E., Lindberg, M. S., & Mednis, A. (2005). Individual quality, survival variation and patterns of phenotypic selection on body condition and timing of nesting in birds. *Oecologia, 143*(3), 365-376.

Bourgeon, S., Le Maho, Y., & Raclot, T. (2009). Proximate and ultimate mechanisms underlying immunosuppression during the incubation fast in female eiders: Roles of triiodothyronine and corticosterone. *General and Comparative Endocrinology, 163*, 77-82.

Bromley, M. (1985). *Wildlife management implications of petroleum exploration and development in wildland environments* . Intermountain Research Station, Ogden, UT. General Technical Report INT-191.

Burton, N. H. K., Rehfisch, M. M., Clark, N. A., & Dodd, S. G. (2006). Impacts of sudden winter habitat loss on the body condition and survival of redshank *Tringa totanus*. *Journal of Applied Ecology, 43*, 464-473.

Clark, R. G., & Nudds, T. D. (1991). Habitat patch size and duck nesting success: The crucial experiments have not been performed. *Wildlife Society Bulletin, 19*(4), 534-543.

Corcoran, R. M., Lovvorn, J. R., Bertram, M. R., & Vivion, M. T. (2007). Lesser scaup nest success and duckling survival on the Yukon Flats, Alaska. *Journal of Wildlife Management, 71*(1), 127-134.

Dawson, R. D., & Clark, R. G. (1996). Effects of variation in egg size and hatching date on survival of lesser scaup *Aythya affinis* ducklings. *IBIS, 138*, 693-699.

Dawson, R. D., & Clark, R. G. (2000). Effects of hatching date and egg size on growth, recruitment, and adult size of lesser scaup. *The Condor, 102*(4), 930-935.

DeVink, J. M., Clark, R. G., Slattery, S. M., & Trauger, D. L. (2008). Are late-spring boreal lesser scaup (*Aythya affinis*) in poor body condition? *The Auk, 125*(2), 291-298.

Esler, D. (1994). Dynamics of ovarian follicles in breeding ducks. *Wilson Bulletin, 106*, 679-688.

Esler, D., Grand, J. B., & Afton, A. D. (2001). Intraspecific variation in nutrient reserve use during clutch formation by lesser scaup. *The Condor, 103*(4), 810-820.

Fast, P. L. F., Clark, R. G., Brook, R. W., & Hines, J. E. (2004). Patterns of wetland use by brood-rearing lesser scaup in the the northern boreal forest of canada *Waterbirds: The International Journal of Waterbird Biology, 27*(2), 177-182.

Forman, R. T. T. (1995). *Land mosaics: The ecology of landscapes and regions*. Cambridge: Cambridge University Press.

Gorman, K. B., Esler, D., Flint, P. L., & Williams, T. D. (2008). Nutrient-reserve dynamics during egg production by female greater scaup (*Aythya marila*): Relationships with timing of reproduction. *The Auk, 125*(2), 384-394.

Groothuis, T. G. G., Muller, W., von Engelhardt, N., Carere, C., & Eising, C. (2005). Maternal hormones as a tool to adjust offspring phenotype in avian species. *Neuroscience and Biobehavioral Reviews, 29*, 392-352.

Gurney, K. E. B., Clark, R. G., Slattery, S. M., Smith-Downey, N. V., Walker, J., Armstrong, L. M., Stephens, S.E., Petrula, M., Corcoran, R.M., Martin, K.H., DeGroot, K.A., Brook, R.W., Afton, A.D., Cutting, K., Warren, J.M., Fournier, M., Koons, D.N. (2011). Time constraints in temperate-breeding species: Influence of growing season length on reproductive strategies. *Ecography, 34*, 628-636.

Gurney, K. E. B., Clark, R. G., & Slattery, S. M. (2012). Seasonal variation in pre-fledgling survival of lesser scaup *Aythya affinis*: Hatch date effects sdepend on maternal body mass. *Journal of Avian Biology, 43*, 68-78.

Hammel, G. S. (1973). The ecology of the lesser scaup (*Aythya affinis-eyton*) in southwestern Manitoba. Unpublished MS Thesis, University of Guelph, Guelph.

Henninger, W. F. (1904). An ornithological reconnoissance of the Grand Reservoir, Ohio, in 1904. *The Wilson Bulletin, 16*(3), 74-76.

Henson, P., & Grant, T. A. (1991). The effects of human disturbance on trumpeter swan breeding behaviour. *Wildlife Society Bulletin, 19*, 248-257.

Horn, D. J., Phillips, M. L., Koford, R. R., Clark, W. R., Sovada, M. A., & Greenwood, R. J. (2005). Landscape composition, patch size, and distance to edges: Interactions affecting duck reproductive success. *Ecological Applications, 15*(4), 1367-1376.

Janczak, A. M., Braastad, B. O., & Bakken, M. (2006). Behavioral effects of embryonic exposure to corticosterone in chickens. *Applied Animal Behaviour Science, 96*, 69-82.

Janczak, A. M., Heikkila, M., Valros, A., Torjesen, P., Anderson, I. L., & Bakken, M. (2007). Effects of embryonic corticosterone exposure and post-hatch handling on tonic immobility and willingness to compete in chicks. *Applied Animal Behaviour Science, 107*, 275-286.

Janczak, A. M., Torjesen, P., Palme, R., & Bakken, M. (2007). Effects of stress in hens on behavior of their offspring. *Applied Animal Behaviour Science, 107*, 66-77.

Johnson, A. L. (1990). Steroidogenesis and actions of steroids in the hen ovary. *CRC Critical Reviews in Poultry Biology, 2*, 319.

Johnson, D. H., & Grier, J. W. (1988). *Determinants of breeding distribution of ducks*. University of Nebraska- Lincoln: USGS Northern Prairie Wildlife Research Center.

Kahl, R. (1991). Boating disturbance of canvasbacks during migration at Lake Poygan, Wisconsin. *Wildlife Society Bulletin, 19*, 242-248.

Keller, V. E. (1991). Effects of human disturbance on eider ducklings *Somateria mollissima* in an estuarine habitat in Scotland. *Biological Conservation, 58*, 213-228.

Kitaysky, A. S., Wingfield, J. C., & Piatt, J. F. (2001). Cortiosterone facilitates begging and affects resource allocation in the black-legged kittiwake. *Behavioral Ecology, 12*(5), 619-625.

Klein, M. L., Humphrey, S. R., & Percival, H. F. (1995). Effects of ecotourism on distribution of waterbirds in a wildlife refuge. *Conservation Biology, 9*(6), 1454-1465.

Kokko, H., & Sutherland, W. J. (2001). Ecological traps in changing environments: Ecological and evolutionary consequences of a behaviorally mediated allee effect. *Evolutionary Ecology Research, 3*, 537-551.

Korschgen, C. E., & Dahlgren, R. B. (1992). 13.2. 15. Human Disturbances of Waterfowl: Causes, Effects, and Management. *Waterfowl Management Handbook*, 12.

Lindeman, D. H., & Clark, R. G. (1999). Amphipods, land-use impacts, and lesser scaup (*Aythya affinis*) distribution in saskatchewan wetlands. *Wetlands, 19*(3), 627-638.

Love, O. P., Wynne-Edwards, K. E., Bond, L., & Williams, T. D. (2008). Determinants of within- and among-clutch variation in yolk corticosterone in the european starling. *Hormones and Behavior, 53*, 104-111.

Lyon, A. G., & Anderson, S. H. (2003). Potential gas development impacts on sage grouse nest initiation and movement. *Wildlife Society Bulletin, 31*(2), 486-491.

Mainguy, J., Bety, J., Gauthier, G., & Giroux, J. F. (2002). Are body condition and reproductive effort of laying greater snow geese affected by the spring hunt? *The Condor, 104*(1), 156-161.

Marini, M. A., Robinson, S. K., & Heske, E. J. (1995). Edge effects on nest predation in the Shawnee National Forest, southern Illinois. *Biological Conservation, 74*, 203-213.

Mazerolle, M. J. (2010). *AICmodavg: Model selection and multimodel inference based on (Q)AIC(c)* in CRAN. Developed for R Development Core Team, . (2010). *R: A language and environment for statistical computing*. Vienna, Austria:

Norton, M. R., Hannon, S. J., & Schmiegelow, F. K. A. (2000). Fragments are not islands: Patch vs landscape perspectives on songbird presence and abundance in a harvested boreal forest. *Ecography, 23*, 209-223.

Paasivaara, A., & Poysa, H. (2007). Survival of common goldeneye *bucephala clangula* ducklings in relation to weather, timing of breeding, brood size, and female condition. *Journal of Avian Biology, 38*, 144-152.

Pasitschniak-Arts, M., Clark, R. G., & Messier, F. (1998). Duck nesting success in a fragmented prairie landscape: Is edge effect important. *Biological Conservation, 85*, 55-62.

Phillips, M. L., Clark, W. R., Nusser, S. M., Sovada, M. A., & Greenwood, R. J. (2004). Analysis of predator movement in prairie landscapes with contrasting grassland composition. *Journal of Mammalogy, 85*(2), 187-195.

R Development Core Team, . (2010). *R: A language and environment for statistical computing*. Vienna, Austria:

Reijnen, R., Foppen, R., & Meeuwsen, H. (1996). The effects of traffic on the density of breeding birds in dutch agricultural grasslands. *Biological Conservation, 75*, 255-260.

Rettenbacher, S., Mostl, E., Hackl, R., & Palme, R. (2005). Corticosterone in chicken eggs. *Annals of the New York Academy of Sciences, 1046*, 193-203.

Rubolini, D., Romano, M., Boncoraglio, G., Ferrari, R. P., Martinelli, R., Galeotti, P., et al. (2005). Effects of elevated egg corticosterone levels on behavior, growth, and immunity of yellow-legged gull (*Larus michahellis*) chicks. *Hormones and Behavior, 47*, 592-605.

Saino, N., Romano, M., Ferrari, R. P., Martinelli, R., & Moller, A. O. (2005). Stressed mothers lay eggs with high corticosterone levels with produce low-quality offspring. *Journal of ExperimentalZoology, 303*(A), 998-1006.

Sargeant, A. B., Greenwood, R. J., Sovada, M. A., & Shaffer, T. L. (1993). *Distribution and abundance of predators that affect duck production- prairie pothole region* No. 194). Washington, D.C: United States Department of the Interior Fish and Wildlife Service Resource Publication.

Schlaepher, M. A., Runge, M. C., & Sherman, P. W. (2002). Ecological and evolutionary traps. *TRENDS in Ecology & Evolution, 17*(10), 474-480.

Schmiegelow, F. K., & Monkkonen, M. (2002). Habitat loss and fragmentation in dynamic landscapes: Avian perspectives from the boreal forest. *Ecological Applications, 12*(2), 375-389.

Siegel, H. S., & Van Kampen, V. (1984). Energy relationships in growing chickens given daily injections of corticosterone. *British Poultry Science, 25*, 477-485.

Siegel, H. S. (1980). Physiological stress in birds. *BioScience, 30*(8), 529-534.

Sorenson, L. G., Nolan, P. M., Brown, A. M., Derrickson, S. R., & Monfort, S. L. (1997). Hormonal dynamics during mate choice in the northern pintail: A test of the "challenge" hypothesis. *Animal Behaviour, 54*, 1117-1133.

Spaans, B., Blijleven, H. J., Popov, I. U., Rykhlikova, M. E., & Ebbinge, B. S. (1998). Dark-bellied brent geese *Branta bernicla bernicla* forego breeding when arctic foxes *Alopexx lagopus* are present during nest initiation. *Ardea, 86*(1), 11-20.

Stephens, S. E., Koons, D. N., Rotella, J. J., & Willey, D. W. (2003). Effects of habitat fragmentation on avian nesting success: A review of the evidence at multiple spatial scales. *Biological Conservation, 115*, 101-110.

Stolen, E. D. (2003). The effects of vehicle passage on foraging behavior of wading birds. *Waterbirds, 26*(4), 429-436.

Talent, L., Jarvis, R., & Krapu, G. (1983). Survival of mallard broods in south-central North Dakota. *The Condor, 85*, 74-78.

Thiel, D., Jenni-Eiermann, S. J., Braunisch, V., Palme, R., & Jenni, L. (2008). Ski tourism affects habitat use and evokes a physiological stress response in capercaille *tetrao urogallus*: A new methodological approach. *Applied Ecology, 45*, 845-853.

Visser, M. E., van Noordwijk, A. J., Tinbergen, J. M., & Lessells, C. M. (1998). Warmer springs lead to mistimed reproduction in great tits. *Proceedings from the Royal Society B, London, 265*, 1867-1870.

Walsh, K. A., Halliwell, D. R., Hines, J. E., Fournier, M. A., Czarnecki, A., & Dahl, M. F. (2006). Effects of water quality on habitat use by lesser scaup (*Aythya affinis*) broods in the boreal Northwest Territories, Canada. *Hydrobiologia, 567*, 101-111.

Wasser, S. K., Bevis, K., King, G., & Hanson, E. (1997). Noninvasive physiological measures of disturbance in the northern spotted owl. *Conservation Biology, 11*(4), 1019-1022.

Wingfield, C. (1994). Modulation of the adrenocortical response to stress in birds. In G. Davey, E. Peter & S. S. Tobe (Eds.), *Perspectives in comparative endocrinology* (pp. 520-528). Ottawa: National Research Council of Canada.

Zegers, D. A., May, S., & Goodrich, L. J. (2000). Identification of nest predation at farm/forest edge and forest interior sites. *Journal of Field Ornithology, 71*(2), 207-216.

Zimpfer N.L., Rhodes, W. E., Silverman, E. D., Zimmerman, G. S., & Koneff, M. D. (2009). *Trends in duck breeding populations, 1955-2009*U.S Fish and Wildlife Service.

3 APPENDIX 1:*A priori* candidate models and exploratory models for Chapter 2

Table 3.1: A complete list of *a priori* exploratory models generated to determine which habitat features (within a 100 m radius from the collection point) and/or biological variables were most strongly correlated OCORT content for female Lesser Scaup collected in Northern Alberta in the summer of 2009.

Model Description
OCORT~1
OCORT~AGRIC
OCORT~ANTHRO
OCORT~BOGSFENS
OCORT~DATE
OCORT~SWAMP
OCORT~TRANSPORT
OCORT~UPLAND
OCORT~WATER
OCORT~WETLAND
OCORT~AGRIC+ANTHRO
OCORT~AGRIC+TRANSPORT
OCORT~AGRIC+WATER
OCORT~AGRIC+WETLAND
OCORT~ANTHRO+WATER
OCORT~BOGSFENS+SWAMP
OCORT~DATE+WATER
OCORT~TRANSPORT+WATER
OCORT~BOGSFENS+SWAMP+WETLAND
OCORT~AGRIC+ANTHRO+BOGSFENS+DATE+SWAMP+UPLAND+WETLAND

Table 3.2: A complete list of a priori exploratory models generated to determine which habitat features (within a 250 m radius from the collection point) and/or biological variables were most strongly correlated to follicular CORT content for female Lesser Scaup collected in Northern Alberta in the summer of 2009.

Model Description
OCORT~1
OCORT~AGRIC
OCORT~ANTHRO
OCORT~BOGSFENS
OCORT~DATE
OCORT~SWAMP
OCORT~TRANSPORT
OCORT~UPLAND
OCORT~WATER
OCORT~WELLS
OCORT~WETLAND
OCORT~AGRIC+ANTHRO
OCORT~AGRIC+TRANSPORT
OCORT~AGRIC+WATER
OCORT~AGRIC+WETLAND
OCORT~ANTHRO+WATER
OCORT~ANTHRO+WELLS
OCORT~BOGSFENS+SWAMP
OCORT~DATE+WATER
OCORT~TRANSPORT+WATER
OCORT~TRANSPORT+WELLS
OCORT~WATER+WELLS
OCORT~AGRIC+ANTHRO+WELLS
OCORT~BOGSFENS+SWAMP+WETLAND
OCORT~AGRIC+ANTHRO+BOGSFENS+DATE+SWAMP+UPLAND+WETLAND+WELLS

Table 3.3: A complete list of *a priori* exploratory models generated to determine which habitat features (within a 500 m radius from the collection point) and/or biological variables were most strongly correlated to follicular CORT content for female Lesser Scaup collected in Northern Alberta in the summer of 2009.

Model Description
OCORT~1
OCORT~AGRIC
OCORT~ANTHRO
OCORT~BOGSFENS
OCORT~DATE
OCORT~SWAMP
OCORT~TRANSPORT
OCORT~UPLAND
OCORT~WATER
OCORT~WELLS
OCORT~WETLAND
OCORT~AGRIC+ANTHRO
OCORT~AGRIC+TRANSPORT
OCORT~AGRIC+WATER
OCORT~AGRIC+WETLAND
OCORT~ANTHRO+WATER
OCORT~ANTHRO+WELLS
OCORT~BOGSFENS+SWAMP
OCORT~DATE+WATER
OCORT~TRANSPORT+WATER
OCORT~TRANSPORT+WELLS
OCORT~WATER+WELLS
OCORT~AGRIC+ANTHRO+WELLS
OCORT~BOGSFENS+SWAMP+WETLAND
OCORT~AGRIC+ANTHRO+BOGSFENS+DATE+SWAMP+UPLAND+WETLAND+WELLS

Table 3.4: A complete list of *a priori* exploratory models generated to determine which habitat features (within a 1500 m radius from the collection point) and/or biological variables were most strongly correlated to follicular CORT content for female Lesser Scaup collected in Northern Alberta in the summer of 2009.

Model Description
OCORT~1
OCORT~AGRIC
OCORT~ANTHRO
OCORT~BOGSFENS
OCORT~DATE
OCORT~SWAMP
OCORT~TRANSPORT
OCORT~UPLAND
OCORT~WATER
OCORT~WELLS
OCORT~WETLAND
OCORT~AGRIC+ANTHRO
OCORT~AGRIC+TRANSPORT
OCORT~AGRIC+WATER
OCORT~AGRIC+WETLAND
OCORT~ANTHRO+WATER
OCORT~ANTHRO+WELLS
OCORT~BOGSFENS+SWAMP
OCORT~DATE+WATER
OCORT~TRANSPORT+WATER
OCORT~WATER+WELLS
OCORT~AGRIC+ANTHRO+WELLS
OCORT~BOGSFENS+SWAMP+WETLAND
OCORT~AGRIC+ANTHRO+BOGSFENS+DATE+SWAMP+UPLAND+WETLAND+WELLS

Table 3.5: A complete list of *a priori* exploratory models generated to determine which habitat features (within a 5000 m radius from the collection point) and/or biological variables were most strongly correlated to follicular CORT content for female Lesser Scaup collected in Northern Alberta in the summer of 2009.

Model Description
OCORT~1
OCORT~AGRIC
OCORT~ANTHRO
OCORT~BOGSFENS
OCORT~DATE
OCORT~SWAMP
OCORT~TRANSPORT
OCORT~UPLAND
OCORT~WATER
OCORT~WELLS
OCORT~WETLAND
OCORT~AGRIC+ANTHRO
OCORT~AGRIC+TRANSPORT
OCORT~AGRIC+WATER
OCORT~AGRIC+WETLAND
OCORT~ANTHRO+WATER
OCORT~ANTHRO+WELLS
OCORT~BOGSFENS+SWAMP
OCORT~DATE+WATER
OCORT~AGRIC+ANTHRO+WELLS
OCORT~BOGSFENS+SWAMP+WETLAND
OCORT~AGRIC+ANTHRO+BOGSFENS+DATE+SWAMP+UPLAND+WETLAND+WELLS

Table 3.6: A complete list of *a priori* exploratory models generated to determine which habitat features (within a 100 m radius from the collection point) were most strongly correlated to body condition (represented by body mass) for female Lesser Scaup collected in Northern Alberta in the summers of 2008 and 2009.

Model Description
BM~1
BM~AGRIC
BM~ANTHRO
BM~BOGSFENS
BM~OCORT
BM~SWAMP
BM~TRANSPORT
BM~UPLAND
BM~WATER
BM~WETLAND
BM~AGRIC+ANTHRO
BM~AGRIC+OCORT
BM~AGRIC+TRANSPORT
BM~AGRIC+WATER
BM~AGRIC+WETLAND
BM~ANTHRO+OCORT
BM~ANTHRO+WATER
BM~BOGSFENS+SWAMP
BM~OCORT+TRANSPORT
BM~OCORT+WATER
BM~TRANSPORT+WATER
BM~AGRIC+ANTHRO+OCORT
BM~AGRIC+ANTHRO+UPLAND
BM~AGRIC+OCORT+TRANSPORT
BM~BOGSFENS+SWAMP+WETLAND
BM~AGRIC+ANTHRO+BOGSFENS+OCORT+SWAMP+UPLAND+WETLAND

Table 3.7: A complete list of *a priori* exploratory models generated to determine which habitat features (within a 250 m radius from the collection point) were most strongly correlated to body mass for female Lesser Scaup collected in Northern Alberta in the summers of 2008 and 2009 (NOCORT= Normalized OCORT).

Model Description
BM~1
BM~AGRIC
BM~ANTHRO
BM~BOGSFENS
BM~NOCORT
BM~SWAMP
BM~TRANSPORT
BM~UPLAND
BM~WATER
BM~WELLS
BM~WETLAND
BM~AGRIC+ANTHRO
BM~AGRIC+NOCORT
BM~AGRIC+TRANSPORT
BM~ AGRIC + WATER
BM~AGRIC+WETLAND
BM~ANTHRO+NOCORT
BM~ANTHRO+WATER
BM~BOGSFENS+SWAMP
BM~NOCORT+WATER
BM~NOCORT+TRANSPORT
BM~TRANSPORT+WATER
BM~TRANSPORT+WELLS
BM~WATER+WELLS
BM~WELLS+NOCORT
BM~AGRIC+ANTHRO+NOCORT
BM~AGRIC+ANTHRO+UPLAND
BM~AGRIC+ANTHRO+WELLS
BM~AGRIC+NOCORT+TRANSPORT
BM~ANTHRO+NOCORT+WELLS
BM~ANTHRO+WELLS+UPLAND
BM~BOGSFENS+SWAMP+WETLAND
BM~AGRIC+ANTHRO+WELLS+NOCORT
BM~AGRIC+ANTHRO+WELLS+UPLAND

BM~ NOCORT+WELLS+TRANSPORT
BM~AGRIC+ANTHRO+BOGSFENS+SWAMP+UPLAND+WETLAND+WELLS+NOCORT

Table 3.8: A complete list of *a priori* exploratory models generated to determine which habitat features (within a 500 m radius from the collection point) were most strongly correlated to body mass for female Lesser Scaup collected in Northern Alberta in the summers of 2008 and 2009.

Model Description
BM~1
BM~AGRIC
BM~ANTHRO
BM~BOGSFENS
BM~OCORT
BM~SWAMP
BM~TRANSPORT
BM~UPLAND
BM~WATER
BM~WELLS
BM~WETLAND
BM~AGRIC+ANTHRO
BM~AGRIC+OCORT
BM~AGRIC+TRANSPORT
BM~ AGRIC + WATER
BM~AGRIC+WETLAND
BM~ANTHRO+OCORT
BM~ANTHRO+WATER
BM~BOGSFENS+SWAMP
BM~OCORT+WATER
BM~OCORT+TRANSPORT
BM~TRANSPORT+WATER
BM~TRANSPORT+WELLS
BM~WATER+WELLS
BM~WELLS+OCORT
BM~AGRIC+ANTHRO+OCORT
BM~AGRIC+ANTHRO+UPLAND
BM~AGRIC+ANTHRO+WELLS
BM~AGRIC+OCORT+TRANSPORT
BM~ANTHRO+OCORT+WELLS
BM~ANTHRO+WELLS+UPLAND
BM~BOGSFENS+SWAMP+WETLAND
BM~AGRIC+ANTHRO+WELLS+OCORT
BM~AGRIC+ANTHRO+WELLS+UPLAND
BM~ OCORT+WELLS+TRANSPORT
BM~AGRIC+ANTHRO+BOGSFENS+SWAMP+UPLAND+WETLAND+WELLS+OCORT

Table 3.9: A complete list of *a priori* exploratory models generated to determine which habitat features (within a 1500 m radius from the collection point) were most strongly correlated to body mass for female Lesser Scaup collected in Northern Alberta in the summers of 2008 and 2009 (NOCORT= Normalized OCORT).

Model Description
BM~1
BM~AGRIC
BM~ANTHRO
BM~BOGSFENS
BM~NOCORT
BM~SWAMP
BM~TRANSPORT
BM~UPLAND
BM~WATER
BM~WELLS
BM~WETLAND
BM~AGRIC+ANTHRO
BM~AGRIC+NOCORT
BM~AGRIC+TRANSPORT
BM~ AGRIC + WATER
BM~AGRIC+WETLAND
BM~ANTHRO+NOCORT
BM~ANTHRO+WATER
BM~BOGSFENS+SWAMP
BM~NOCORT+WATER
BM~NOCORT+TRANSPORT
BM~TRANSPORT+WATER
BM~WATER+WELLS
BM~WELLS+NOCORT
BM~AGRIC+ANTHRO+NOCORT
BM~AGRIC+ANTHRO+UPLAND
BM~AGRIC+ANTHRO+WELLS
BM~AGRIC+NOCORT+TRANSPORT
BM~ANTHRO+NOCORT+WELLS
BM~ANTHRO+WELLS+UPLAND
BM~BOGSFENS+SWAMP+WETLAND
BM~AGRIC+ANTHRO+WELLS+NOCORT
BM~AGRIC+ANTHRO+WELLS+UPLAND
BM~AGRIC+ANTHRO+BOGSFENS+SWAMP+UPLAND+WETLAND+WELLS+NOCORT

Table 3.10: A complete list of *a priori* exploratory models generated to determine which habitat features (within a 5000 m radius from the collection point) were most strongly correlated to body mass for female Lesser Scaup collected in Northern Alberta in the summers of 2008 and 2009.

Model Description
BM~1
BM~AGRIC
BM~ANTHRO
BM~BOGSFENS
BM~OCORT
BM~SWAMP
BM~TRANSPORT
BM~UPLAND
BM~WATER
BM~WELLS
BM~WETLAND
BM~AGRIC+ANTHRO
BM~AGRIC+OCORT
BM~AGRIC+TRANSPORT
BM~ AGRIC + WATER
BM~AGRIC+WETLAND
BM~ANTHRO+OCORT
BM~ANTHRO+WATER
BM~BOGSFENS+SWAMP
BM~OCORT+WATER
BM~OCORT+TRANSPORT
BM~WELLS+OCORT
BM~AGRIC+ANTHRO+OCORT
BM~AGRIC+ANTHRO+UPLAND
BM~AGRIC+ANTHRO+WELLS
BM~AGRIC+OCORT+TRANSPORT
BM~ANTHRO+OCORT+WELLS
BM~ANTHRO+WELLS+UPLAND
BM~BOGSFENS+SWAMP+WETLAND
BM~AGRIC+ANTHRO+WELLS+OCORT
BM~AGRIC+ANTHRO+WELLS+UPLAND
BM~AGRIC+ANTHRO+BOGSFENS+SWAMP+UPLAND+WETLAND+WELLS+OCORT

Table 3.11: A complete list of *a priori* exploratory models generated to determine which habitat features (within a 100 m radius from the collection point) were most strongly correlated to determine nest initiation date for female Lesser Scaup collected in Northern Alberta in the summers of 2008 and 2009.

Model Description
NID~1
NID~AGRIC
NID~ANTHRO
NID~BOGSFENS
NID~OCORT
NID~NBM
NID~SWAMP
NID~TRANSPORT
NID~UPLAND
NID~WATER
NID~WETLAND
NID~AGRIC+ANTHRO
NID~AGRIC+OCORT
NID~AGRI+NBM
NID~AGRIC+TRANSPORT
NID~AGRIC+WATER
NID~AGRIC+WETLAND
NID~ANTHRO+OCORT
NID~ANTHRO+NBM
NID~ANTHRO+WATER
NID~OCORT+NBM
NID~OCORT+WATER
NID~TRANSPORT+OCORT
NID~TRANSPORT+WATER
NID~AGRIC+ANTHRO+OCORT
NID~AGRIC+ANTHRO+NBM
NID~AGRIC+TRANSPORT+OCORT
NID~AGRIC+TRANSPORT+NBM
NID~BOGSFENS+SWAMP+WETLAND
NID~AGRIC+ANTHRO+BOGSFENS+OCORT+NBM+SWAMP+UPLAND+WETLAND

Table 3.12: A complete list of *a priori* exploratory models generated to determine which habitat features (within a 250 m radius from the collection point) were most strongly correlated to determine nest initiation date for female Lesser Scaup collected in Northern Alberta in the summers of 2008 and 2009.

Model Description
NID~1
NID~AGRIC
NID~ANTHRO
NID~BOGSFENS
NID~OCORT
NID~BM
NID~SWAMP
NID~TRANSPORT
NID~UPLAND
NID~WATER
NID~WELLS
NID~WETLAND
NID~AGRIC+ANTHRO
NID~AGRIC+OCORT
NID~AGRIC+BM
NID~AGRIC+TRANSPORT
NID~AGRIC+WATER
NID~AGRIC+WETLAND
NID~ANTHRO+OCORT
NID~ANTHRO+BM
NID~ANTHRO+WATER
NID~BOGSFENS+SWAMP
NID~OCORT+BM
NID~OCORT+WATER
NID~TRANSPORT+OCORT
NID~TRANSPORT+WATER
NID~TRANSPORT+WELLS
NID~WATER+WELLS
NID~WELLS+OCORT
NID~AGRIC+ANTHRO+OCORT
NID~AGRIC+ANTHRO+BM
NID~AGRIC+TRANSPORT+OCORT
NID~AGRIC+TRANSPORT+BM
NID~ANTHRO+OCORT+WELLS

NID~ANTHRO+BM+WELLS
NID~BOGSFENS+SWAMP+WETLAND
NID~TRANSPORT+WELLS+OCORT
NID~TRANSPORT+WELLS+BM
NID~AGRIC+ANTHRO+WELLS+OCORT
NID~AGRIC+ANTHRO+WELLS+BM
NID~AGRIC+ANTHRO+WELLS+UPLAND
NID~AGRIC+ANTHRO+BOGSFENS+OCORT+BM+SWAMP+UPLAND+WETLAND+WELLS

Table 3.13: A complete list of *a priori* exploratory models generated to determine which habitat features (within a 500 m radius from the collection point) were most strongly correlated to determine nest initiation date for female Lesser Scaup collected in Northern Alberta in the summers of 2008 and 2009.

Model Description
NID~1
NID~AGRIC
NID~ANTHRO
NID~BOGSFENS
NID~OCORT
NID~BM
NID~SWAMP
NID~TRANSPORT
NID~UPLAND
NID~WATER
NID~WELLS
NID~WETLAND
NID~AGRIC+ANTHRO
NID~AGRIC+OCORT
NID~AGRIC+BM
NID~AGRIC+TRANSPORT
NID~AGRIC+WATER
NID~AGRIC+WETLAND
NID~ANTHRO+OCORT
NID~ANTHRO+BM
NID~ANTHRO+WATER
NID~BOGSFENS+SWAMP
NID~OCORT+BM
NID~OCORT+WATER
NID~TRANSPORT+OCORT
NID~TRANSPORT+WATER
NID~TRANSPORT+WELLS
NID~WATER+WELLS
NID~WELLS+OCORT
NID~AGRIC+ANTHRO+OCORT
NID~AGRIC+ANTHRO+BM
NID~AGRIC+TRANSPORT+OCORT
NID~AGRIC+TRANSPORT+BM
NID~ANTHRO+OCORT+WELLS

NID~ANTHRO+BM+WELLS
NID~BOGSFENS+SWAMP+WETLAND
NID~TRANSPORT+WELLS+OCORT
NID~TRANSPORT+WELLS+BM
NID~AGRIC+ANTHRO+WELLS+OCORT
NID~AGRIC+ANTHRO+WELLS+BM
NID~AGRIC+ANTHRO+WELLS+UPLAND
NID~AGRIC+ANTHRO+BOGSFENS+OCORT+BM+SWAMP+UPLAND+WETLAND+WELLS

Table 3.14: A complete list of *a priori* exploratory models generated to determine which habitat features (within a 1500 m radius from the collection point) were most strongly correlated to determine nest initiation date for female Lesser Scaup collected in Northern Alberta in the summers of 2008 and 2009.

Model Description
NID~1
NID~AGRIC
NID~ANTHRO
NID~BOGSFENS
NID~OCORT
NID~BM
NID~SWAMP
NID~TRANSPORT
NID~UPLAND
NID~WATER
NID~WELLS
NID~WETLAND
NID~AGRIC+ANTHRO
NID~AGRIC+OCORT
NID~AGRIC+BM
NID~AGRIC+TRANSPORT
NID~AGRIC+WATER
NID~AGRIC+WETLAND
NID~ANTHRO+OCORT
NID~ANTHRO+BM
NID~ANTHRO+WATER
NID~BOGSFENS+SWAMP
NID~OCORT+BM
NID~OCORT+WATER
NID~TRANSPORT+OCORT
NID~TRANSPORT+WATER
NID~WATER+WELLS
NID~WELLS+OCORT
NID~AGRIC+ANTHRO+OCORT
NID~AGRIC+ANTHRO+BM
NID~AGRIC+TRANSPORT+OCORT
NID~AGRIC+TRANSPORT+BM
NID~ANTHRO+OCORT+WELLS
NID~ANTHRO+BM+WELLS

NID~BOGSFENS+SWAMP+WETLAND
NID~AGRIC+ANTHRO+WELLS+OCORT
NID~AGRIC+ANTHRO+WELLS+BM
NID~AGRIC+ANTHRO+WELLS+UPLAND
NID~AGRIC+ANTHRO+BOGSFENS+OCORT+BM+SWAMP+UPLAND+WETLAND+WELLS

Table 3.15: A complete list of *a priori* exploratory models generated to determine which habitat features (within a 5000 m radius from the collection point) were most strongly correlated to determine nest initiation date for female Lesser Scaup collected in Northern Alberta in the summers of 2008 and 2009.

Model Description
NID~1
NID~AGRIC
NID~ANTHRO
NID~BOGSFENS
NID~OCORT
NID~BM
NID~SWAMP
NID~TRANSPORT
NID~UPLAND
NID~WATER
NID~WELLS
NID~WETLAND
NID~AGRIC+ANTHRO
NID~AGRIC+OCORT
NID~AGRIC+BM
NID~AGRIC+TRANSPORT
NID~AGRIC+WATER
NID~AGRIC+WETLAND
NID~ANTHRO+OCORT
NID~ANTHRO+BM
NID~ANTHRO+WATER
NID~BOGSFENS+SWAMP
NID~OCORT+BM
NID~OCORT+WATER
NID~TRANSPORT+OCORT
NID~WELLS+OCORT
NID~AGRIC+ANTHRO+OCORT
NID~AGRIC+ANTHRO+BM
NID~AGRIC+TRANSPORT+OCORT
NID~AGRIC+TRANSPORT+BM
NID~ANTHRO+OCORT+WELLS
NID~ANTHRO+BM+WELLS
NID~BOGSFENS+SWAMP+WETLAND
NID~AGRIC+ANTHRO+WELLS+OCORT

NID~AGRIC+ANTHRO+WELLS+BM
NID~AGRIC+ANTHRO+WELLS+UPLAND
NID~AGRIC+ANTHRO+BOGSFENS+OCORT+BM+SWAMP+UPLAND+WETLAND+WELLS

4 APPENDIX 2: Additional Figures

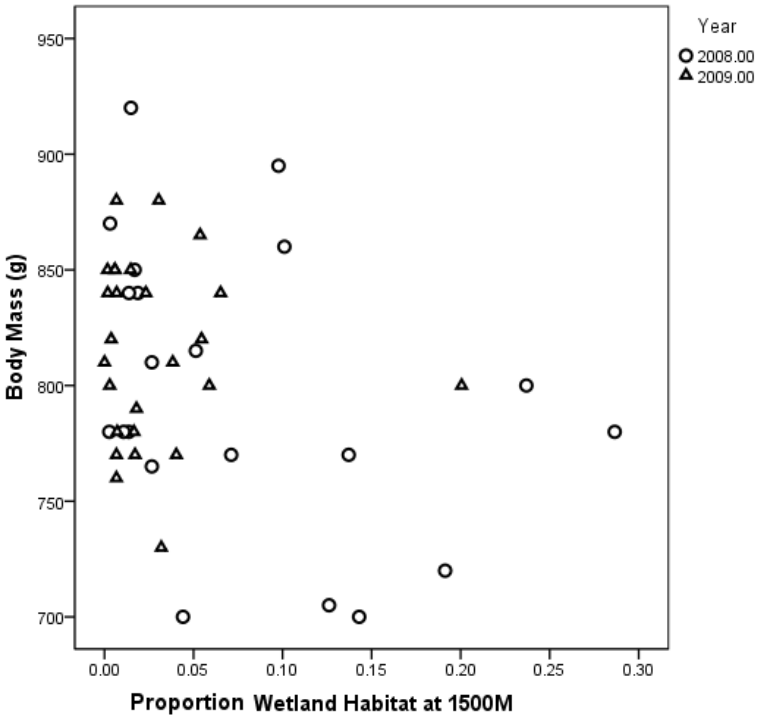

Figure 4.1: Relationship between adult female lesser scaup (*Aythya affinis*) body mass and proportion of wetland habitat within a 1500 m radius of the collection site, for birds collected in the northern Alberta, 2008 and 2009. Collection year had no significant impact on the relationship between body mass (ANCOVA, F=0.19, p=0.67). There was no significant interaction between wetland habitat at the 1500 m buffer zone and collection year (ANOVA, F=1.12, p= 0.55)

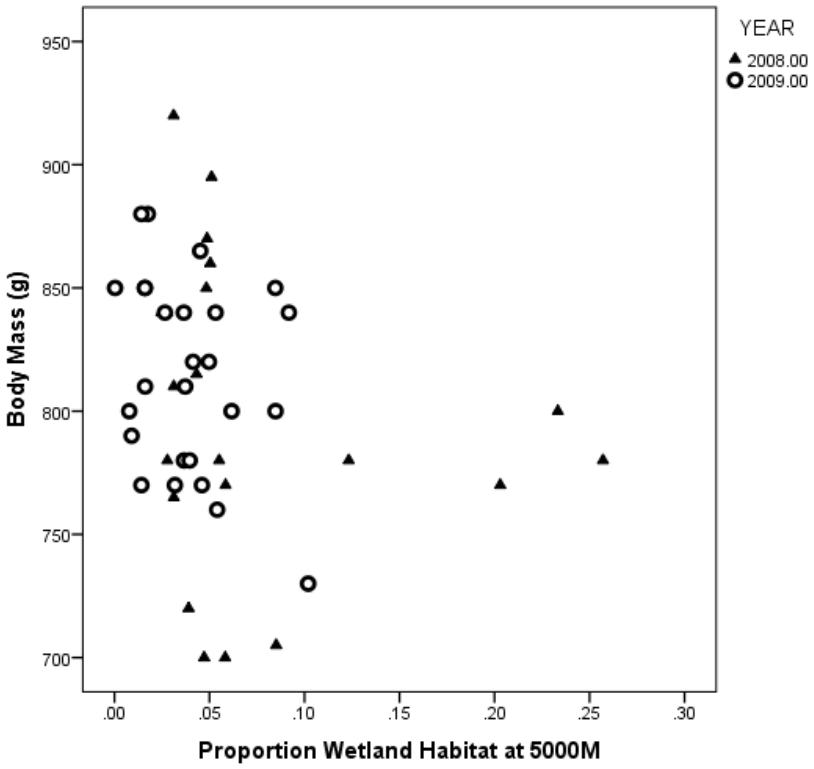

Figure 4.2: Relationship between adult female lesser scaup (*Aythya affinis*) body mass and proportion of wetland habitat within a 5000 m radius of the collection site, for birds collected in the northern Alberta, 2008 and 2009. Collection year had no significant impact on the relationship between body mass and percentage of wetland habitat (ANCOVA, f=0.45, p=0.51). There was no significant interaction between wetland habitat at the 5000 m buffer zone and collection year (ANOVA, F=5.34, p= 0.17)

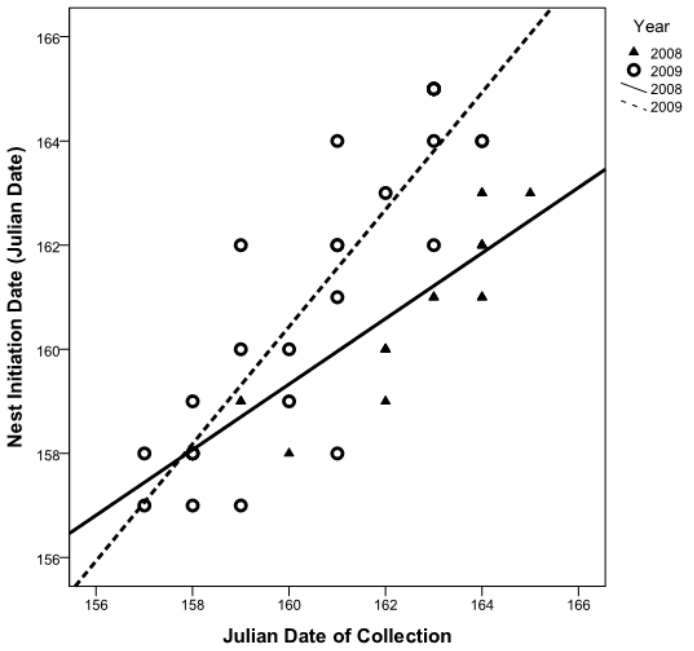

Figure 4.3: Relationship between nest initiation and collection dates (both represented as days since 1 January) for female lesser scaup (*Aythya affinis*) collected in northern Alberta, 2008 and 2009. Regression analysis revealed a significant positive relationship between nest initiation and collection dates in 2008 (R^2= 0.70, F=44.12, p<0.00, SE= 0.09) and 2009 (R^2= 0.76, F=80.38, p<0.00, 0.09). Pooled data revealed a non-significant relationship between nest initiation date and date (R^2= 0.56, F=58.80, p=0.00). There was a significant interaction between collection date and collection year (ANOVA, F=13.35, p=<0.00).

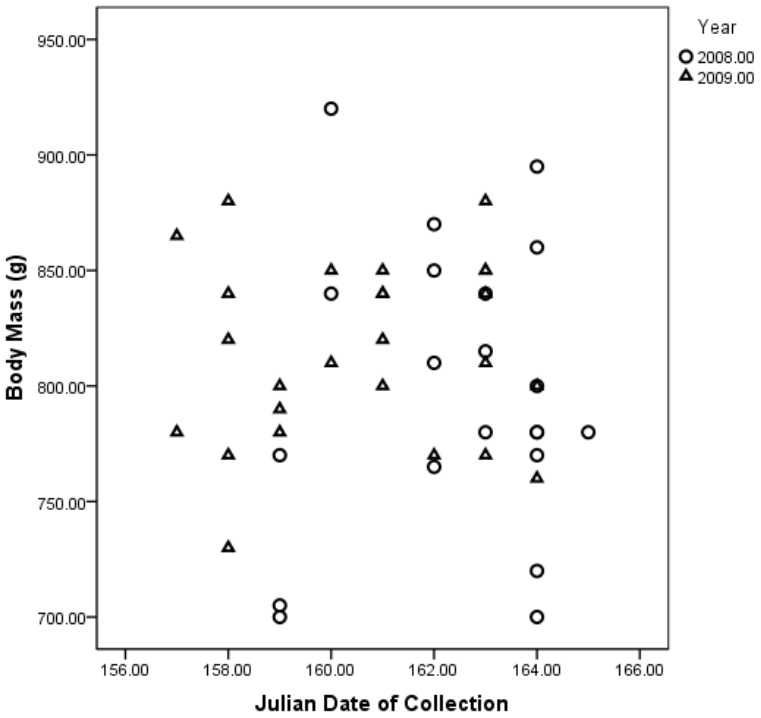

Figure 4.4: Relationship between body mass and collection dates (represented as days since 1 January) for female lesser scaup (*Aythya affinis*) collected in northern Alberta, 2008 and 2009. Regression analysis revealed a non-significant relationship between body mass and collection dates in 2008 (R^2=-0.05, F=0.08, p= 0.77) and 2009 (R^2= 0.04, F=0.08, p=0.77). Pooled data revealed a non-significant relationship between body mass and collection date (R^2= -0.02, F=0.01, p=0.91). There was no significant interaction between collection year and collection date (ANOVA, F=1.74, p=0.12).

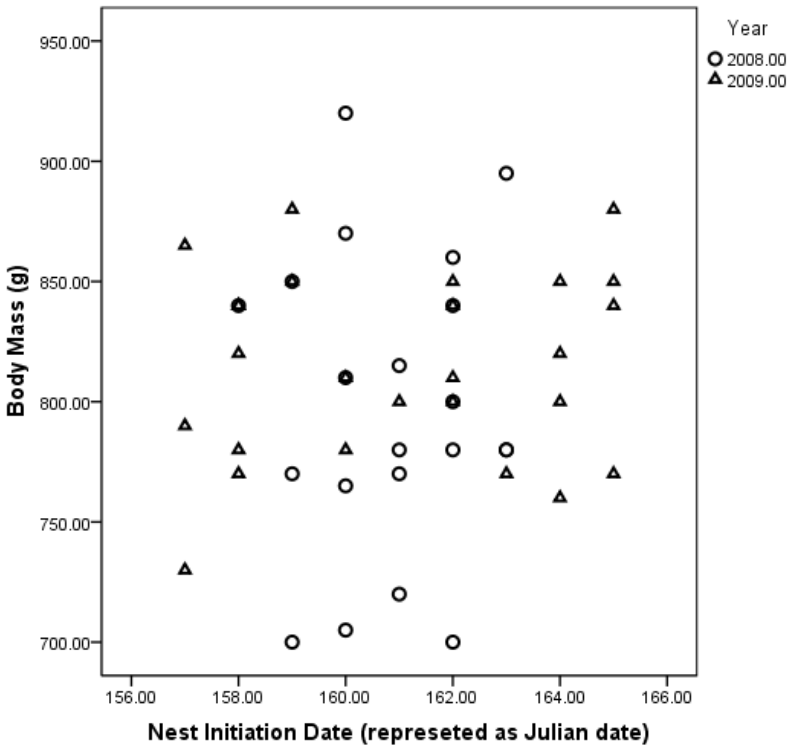

Figure 4.5: Relationship between body mass and nest initiation dates (represented as days since 1 January) for female lesser scaup (*Aythya affinis*) collected in northern Alberta, 2008 and 2009. Regression analysis revealed a non-significant relationship between body mass and nest initiation date in 2008 (R^2=0.001, F=0.016, p=0.90) and 2009 (R^2= 0.014, F=0.08, p=0.57). Pooled data revealed a non-significant relationship between body mass and collection date (R^2=0.006, F=0.27, p=0.61). There was no significant interaction between collection year and nest initiation date (ANOVA, F=0.38, p=0.94).

5 APPENDIX 3: Metabolomic analyses of lesser scaup (*Aytha affinis*) plasma collected from two areas of varying disturbance in the boreal forests of Alberta

5.1 Introduction:

The lesser scaup (*Aythya affinis*, henceforth scaup) population decreased during the 1980s and has remained below conservation objectives. (Austin et al., 2000; Austin et al., 2006; Zimpfer et al., 2009). With 67% of the breeding population nesting in the boreal forest habitats of Canada (Afton & Anderson, 2001), understanding the factors in this habitat that may impact reproduction is vital to successful development of wildlife management decisions. Female scaup are highly philopatric species (i.e., return to their hatch place to breed and raise young (Afton, 1984; Johnson & Grier, 1988)). Unfortunately, anthropogenic activities have significantly altered the boreal forest landscape (Bayne & Hobson, 1998). Nesting female scaup may thus be situated near these altered landscapes, which may stress them during the critical reproduction period

The field of metabolomics evaluates the occurrence of natural, low-weight endogenous metabolites that arise during, or at the conclusion of metabolism (Fiehn, 2002; Ramsden, 2009). An organism's metabolome (Fiehn, 2002; Miller, 2007; Rozen et al., 2005; Viant, Rosenblum, & Tjeerdema, 2003; Viant, 2003) is a composite of the metabolites found in the sample and is the result of the interaction of the organism's genetic make-up with the numerous factors found in its environment (Rochfort, 2005). The objective of this preliminary study was to compare metabolome profiles of female scaup collected in two different areas of anthropogenic disturbance (oil and gas versus agriculture), and to determine whether metabolomes were influenced by habitat.

5.2 Methods:

Female scaup were collected by shooting in early-to-mid June (early-laying period) in the western boreal forests of Northern Alberta in 2008 and 2009 between the general vicinities of Utikuma Lake (55° 52′ 0″ N, 115° 25′ 0″ W) and east towards Lac La Biche (54° 46′ 7″ N, 111° 58′ 50″ W), Alberta. Scaup were further classified as coming from one ofs two habitats of different anthropogenic disturbance: 1) habitat characterized by permanent removal of the boreal forest, sustained by the presence of hay fields and pasture (in areas between Athabasca east to Cold Lake); and 2) habitat impacted by the oil and gas industry (in areas between Utikuma west to Slave Lake). Upon collection, location was recorded using a hand-held GPS device (Garmin GPSMAP 76CSx). Blood samples were collected into heparinised plasma separator tubes (originally BD Hemoguard™ Closure Vacutainers (discontinued) then BD Vacutainer PST™ Gel and Lithium Heparin tubes in 2009) by collecting blood from the jugular vein, carotid arteries, and the heart. The blood was centrifuged for 10 mins at 2000 rpm, and kept on ice until frozen/storage in a -80^{o}C freezer.

Ten samples (5 from oil and gas predominated habitat and 5 from agriculture predominated habitat) were randomly selected after an initial visual screening for spectra quality, and prepared/subjected to 1H-NMR Spectroscopy. A volume of 100 uL plasma was diluted with 500 uL of a 0.1 M sodium phosphate buffer solution (pH 7.0, made with 99.9% deuterium oxide obtained from Cambridge Isotope Laboratories Inc., Andover, MA) containing 1 mM sodium-3-trimethyl-silyl-[2,2,3,3-d_4] propionate (TMSP, also obtained from Cambridge Isotope Laboratories Inc., Andover, MA) as an internal standard. Samples were placed in 5mm NMR tubes (509 UP tubes from Norell, Landisville, NJ) and subjected to ^{1}H NMR using a Bruker 500

MHz Avance NMR spectrometer with an Inverse Triple Resonance Probe (TXI, 5 mm) at the Saskatchewan Structural Sciences Center (SSSC) in Saskatoon, Saskatchewan. Water suppression using excitation sculpting with gradients (Hwang & Shaka, 1995) pulse sequence was used for each sample to suppress the water peak in the spectral data. A total of 32 free induction decays, were accumulated over a spectral width of 6.51 kHz and a temperature of 297.0 K. Acquisition time was 5.03 seconds with a relaxation delay of 1.000 seconds with an experimental time of 3.22 minutes.

The NMR spectra were manually phased and baseline corrected to the referenced TMSP (δ 0.00) internal standard using XWINNMR (Version 3.5, Bruker, Germany). Analysis of metabolite composition was performed utilizing the PRIMER software package (Clarke & Warwick, 2001). Relative metabolite abundances were first square-root transformed, which results in down-weighting metabolites of high relative abundance, and also causes less abundant metabolites to be competitive in similarity analysis (Chang & Goldberg, 1978; Clarke, 1993; Clarke & Warwick, 2001). Bray-Curtis calculations were then performed on the transformed data, which computes a similarity coefficient comparing the metabolite profiles of two birds. If the metabolite composition between two birds is completely different the similarity coefficient is zero, whereas birds with identical metabolite profiles have a similarity coefficient of 100 (Clarke & Warwick, 2001) . The results from the Bray-Curtis computation were ranked from lowest (indicating high similarity) to highest (indicating low similarity) in a similarity matrix, and used to create an ordination plot (graphical representation) with non-metric multidimensional scaling (MDS) (Clarke & Green, 1988; Clarke & Warwick, 2001). The MDS algorithm results in the continual placement and re-placement of each point, until the ordination plot most accurately

represents how dissimilar the metabolme profiles are for all individuals (Clarke & Warwick, 2001).

The analysis of similarities (ANOSIM) test utilizes the similarity matrix previously discussed to compare similarities among individuals within a collection area, against similarities of individuals between collection areas (Clarke & Green, 1988; Clarke & Warwick, 2001). The resulting value indicates whether metabolite composition varied significantly between the two collection areas. Similarity percentages (SIMPER) analysis, which assesses the contribution of each metabolite and how it relays to the difference observed between collection sites, was performed to determine which metabolites contributed most to differences between habitat types (Clarke & Warwick, 2001).

5.3 Results

Generated metabolite profiles using nonmetric MDS are depicted in Figure 5.1. One bird (58S) was removed because it was significant outlier. Metabolite profiles differed between birds collected in oil and gas versus agriculture dominated habitats with 10 metabolites accounting for 15.6% of the dissimilarity between the two groups (Table 5.1). Of these 10 metabolites, isoleucine contributed most to the dissimilarity between birds collected in oil and agriculture-dominated habitats (5.03%), while 2-hydroxybutyric acid and glucose contributed second and third most (2.84% and 2.64% respectively). An example of a comparison between birds collected in shown in Figure 5.2 (R=0.24, p=0.10, ANOSIM).

5.4 Discussion:

Metabolomic analysis revealed differences in plasma profiles between birds collected in two habitats with contrasting human disturbances. Anthropogenic disturbances have negatively impacted a wide range of avian fauna (Barry, Spencer, & Canadian Wildlife Service, 1976; Blas et al., 2007; Bromley, 1985; Walker, Naugle, & Doherty, 2007) , and may be perceived as potential stressors. When faced with these stressors, the release of corticosterone (or CORT) in birds facilitate a number of behavioural and physiological changes that allow the individual to either cope with the stressor, or escape (Sapolsky, Romero, & Munck, 2000). The physiological changes that accompany the stress response can result in a myriad of changes to an individuals' metabolome. The top 10 metabolites which explain 15.6% of the difference between habitats play varying roles in both the stress response and metabolism, including the gluceoneogenic pathway (resulting in the formation of glucose) or the glycolytic pathway (resulting in the generation of energy in the form of ATP) (Brown & Poon, 2005)

Isoleucine was the most important metabolite distinguishing birds collected in oil and gas from agriculture predominated habitats. Isoleucine and other branched chain amino acids (BCAA, also including leucine and valine) are utilized in protein synthesis and can also be used to generate precursors and compounds for utilization in gluconeogenesis and the tricarboxylic acid cycle (TCA) (Chang & Goldberg, 1978; Harper, Miller, & Block, 1984). Isoleucine plays a role in providing energy to help restore homeostasis in individuals who have experienced stress, trauma, or starvation (Dadmarz et al., 1998; Milakofsky et al., 1985; Ni et al., 2008; Teague et al., 2007). Sprague-Dawley rats experienced elevated levels of isoleucine following acute stress (Teague et al., 2007). Isoleucine's primary role in gluconeogenesis suggests a stronger role in the

acute stress response, however isoleucine may also play a role in chronic stress. Broiler chickens (*Gallus domesticus*) had moderately high abundances of isoleucine after 28 days of low and high intensity lighting regimes, while birds exposed to intermediate lighting regimes displayed the opposite trend with elevated levels of isoleucine on days 14 and 21 of the experiment, but decreased levels of isoleucine on day 28 (Premkumar, 2012). The higher relative abundance of isoleucine in birds collected from agriculture predominated habitats may indicate a higher stress response compared to birds collected from oil and gas predominated habitat. Alternatively, the higher relative abundance of isoleucine may also indicate the presence of chronic stressors in habitats predominated by agriculture habitats.

At the onset of the stress response; CORT causes the mobilization of stored reserves in exercising muscle, while at the same time inhibiting further energy storage by the body (Sapolsky et al., 2000). Gluconeogenesis is initiated in order to increase circulating blood glucose levels in order to supply the body with the materials required for the individual to face the stressor, or flee (Siegel, 1980). Elevated glucose in scaup may be expected due to acute stress as a result of the collection method, but whether glucose concentrations could also be attributable to chronic stressors caused by surrounding habitat is difficult to ascertain, and a limitation of this study. Glucose levels in breeding blue-winged teal (*Anas discors*) hens were found to be responsive to CORT supplementation, as well as to fasting, a chronic stressor (Harris, 1970). Some evidence suggests that exposure to chronic stressors may down-regulate the stress response, depressing baseline levels of CORT, or reducing CORT release in the face of stressors (Cyr & Romero, 2007; Rich & Romero, 2004; Walker, Dee Boersma, & Wingfield, 2006). If this is the case, corresponding glucose abundances in this study could be indicative of either chronic or acute stress, depending on how the individual responds to CORT release.

2-Hydroxybutyric acid is a metabolite that arises from the catabolism of amino acids (Landaas & Petersen, 1975). During extreme physical exertion, anaerobic metabolism leads to an increased concentration of lactate as a result of carbohydrate metabolism. Lactate dehydrogenase, pyruvate dehydrogenase, and the oxidizing and reducing agents NAD/$NADH_2$ play dual roles in the formation and degradation of lactic acid and 2-hydroxybutyric acid (Landaas & Petersen, 1975). Thus, production of 2-hydroxybutyric acid is positively correlated to the production of lactate (Landaas & Petersen, 1975), which may result from either chronic or acute stress.

Glycolysis is usually an aerobic process resulting in the production of pyruvate, which is typically converted into acetyl coenzyme A (Acetyl CoA) for use in the TCA cycle (Brown & Poon, 2005). However in the absence of oxygen, anaerobic respiration must occur for the continued generation of energy (Brown & Poon, 2005). The reduction of pyruvate to lactate under anaerobic conditions allows for the regeneration of NAD+ required for glycolysis, although an increase in lactate buildup is also associated with muscle fatigue due to the formation of lactic acid (Brown & Poon, 2005). Exposure to elevated CORT concentrations have been reported to exhaust aerobic metabolism in broiler chickens subjected to capture stress, resulting in the activation of anerobic respiration, and causing lactate buildup through the reduction of pyruvate (Nijdam et al., 2005). Young adult Sprague-Dawley rats subjected to chronic stress over a period of 44 days showed increased levels of plasma lactate on day 9, however a decrease in plasma lactate was also seen with rats exposed to acute stress (Teague et al., 2007). Male Landaise-strain geese exhibited elevated levels of plasma lactate within 2 minutes of handling stress (Maho et al., 1992). Broiler chickens subjected to capture stress not only had significantly elevated levels of plasma lactate at the beginning of the procedure, but

plasma lactate levels remained elevated during transportation, and until slaughter (Nijdam et al., 2005). Lactate thus can be produced in response to both acute and chronic stress, and distinguishing the source of elevated concentrations may not be possible.

Choline is an essential dietary amine (Blusztajn, 1998) and is an important precursor for many compounds required for physiological function. In the absence of methionine, choline can provide a source of methyl groups which can be utilized in the biosynthesis of the amino acid creatine (Stevens, 1996), which is stored and utilized in muscle metabolism (Casey et al., 1996). Choline is also a precursor for the neurotransmitter acetylcholine (Blusztajn, 1998), which is both synthesized and utilized in the peripheral and central nervous systems (Feldberg & Vogt, 1948). Sprague-Dawley rats subjected to restraint stress over a period of 44 days exhibited elevated choline levels at the conclusion of the experiment (Teague et al., 2007), an indication of chronic stress.

Preliminary analysis has revealed that subtle differences may exist between birds living in different environmental conditions. Increased concentrations of certain metabolites (i.e., isoleucine and choline) in birds collected in agriculture predominated sites may be indicative of chronic stressors in these habitats. Further analysis is required before conclusions can be drawn.

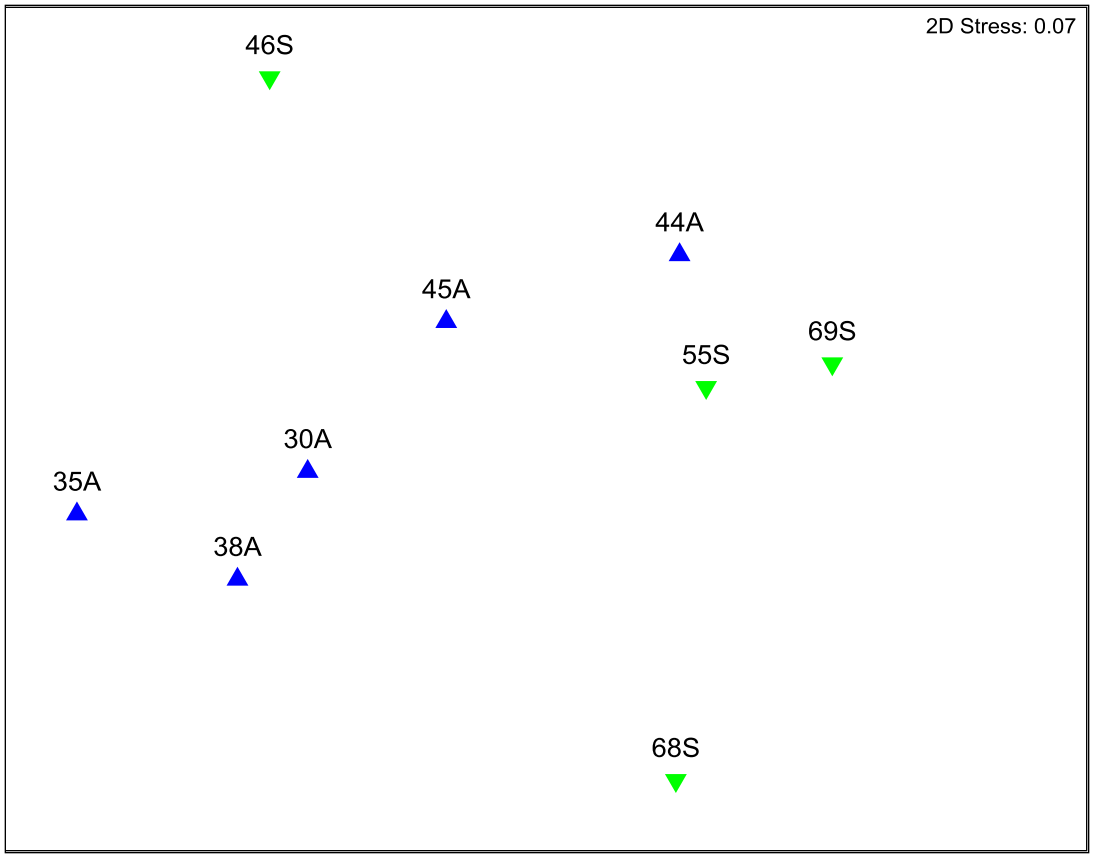

Figure 5.1: Comparison of 9 female lesser scaup collected in 2008 (one outlier eliminated) from two regions of Alberta representing differing levels of disturbance. Individuals labelled with A were collected from agriculture-dominated habitats, while individuals labelled with S were collected from oil/gas-dominated habitats. The nonmetric multidimensional scaling (NMDS) represents 383 metabolites and is based on square-root transformed standardized abundances and Bray-Curtis similarities (stress = 0.13). Individuals with very similar metabolomes are placed close together in space, while individuals with dissimilar metabolomes are placed further apart.

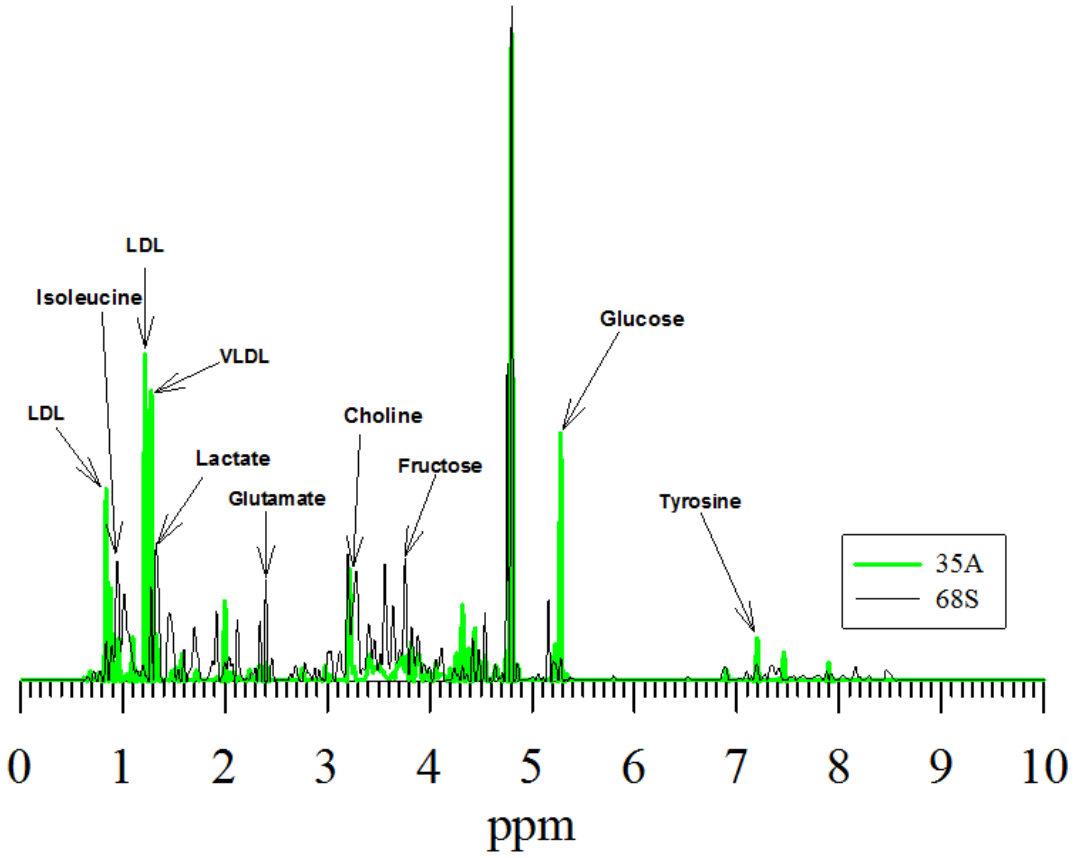

Figure 5.2: Comparison of 2 female lesser scaup (35A from agriculture-dominated habitat, and 68S from oil-dominated habitat) spectra metabolite abundance profiles with some metabolites labelled.

Table 5.1: List of the top 10 metabolites that best distinguish between scaup plasma collected in agriculture vs. oil-based habitats for birds in the MDS plot/ordination. These metabolites explain 15.6% of the difference between the dissimilarity between the two groups. Average dissimilarity is the contribution of the dissimilarity from each metabolite, for all pairs, between group samples. Average dissimilarity SD is the standard deviation value of the average dissimilarity.

Metabolite Identity	Metabolite ppm	Athabasca	Slave Lake	Average Dissimilarity	Average Dissimilarity SD	% Contribution	Cumulative Contribution %
		SQRT Average Abundance	SQRT Average Abundance				
Isoleucine	1.28	2.84	1.78	1.47	1.786	3.06	3.06
Isoleucine	1.22	1.79	0.56	0.94	1.38	1.96	5.03
2 hydroxybutyric acid	0.84	1.85	0.54	0.86	1.52	1.79	6.82
Lactate	1.3	0.89	1.47	0.81	1.12	1.70	8.51
Glucose	5.32	0.56	1.41	0.71	1.06	1.49	10.00
Unknown	4.32	1.08	0.34	0.57	1.38	1.18	11.17
Glucose	5.30	1.00	0.00	0.55	0.79	1.15	12.33
Unknown	4.46	1.12	1.01	0.54	1.56	1.13	13.46
Choline	3.22	1.42	0.50	0.53	1.46	1.11	14.57
2 hydroxybutyric acid	0.88	2.10	2.10	0.50	1.73	1.05	15.62

5.5 References

Afton, A. D. (1984). Influence of age and time on reproductive performance of female lesser scaup. *The Auk, 101*, 255-265.

Afton, A. D., & Anderson, M. G. (2001). Declining scaup populations: A retrospective analysis of long-term population and harvest survey data. *Journal of Wildlife Management, 65*(4), 781-796.

Austin, J. E., Afton, A. D., Anderson, M. G., Clark, R. G., Custer, C. M., Lawrence, J. S., et al. (2000). Declining scaup populations: Issues, hypotheses, and research needs. *Wildlife Society Bulletin, 28*(1), 254-263.

Austin, J. E., Anteau, M. J., Barclay, J. S., Boomer, G. S., Rohwer, F. C., & Slattery, S. M. (2006). Declining scaup populations: Reassessment of the issues, hypotheses, and research directions. *Second Scaup Workshop*, Bismarck, ND.

Barry, T. W., & Spencer, R. (1976). *Wildlife response to oil well drilling*. Ottawa, ON: Canadian Wildlife Service.

Barry, T. W., Spencer, R., & Canadian Wildlife Service. (1976). *Wildlife response to oil well drilling* Progress Notes. Ottawa: Canadian Wildlife Service.

Bayne, E. M., & Hobson, K. A. (1998). The effects of habitat fragmentation by forestry and agriculture on the abundance of small mammals in the southern boreal mixedwood forest. *Canadian Journal of Zoology, 76*, 62-69.

Blas, J., Bortolotti, G. R., Tella, J. L., Baos, R., & Marchant, T. A. (2007). Stress response during development predicts fitness in a wild, long lived vertebrate. *Proceedings of the Royal Society of London, 104*(21), 8880-8884.

Blusztajn, J. K. (1998). Choline, a vital amine. *Science, 281*(5378), 794-795.

Bromley, M. (1985). *Wildlife management implications of petroleum exploration and development in wildland environments* No. Technical Report). Ogden,UT,USA: Forest Service.

Brown, W., & Poon, T. (2005). *Introduction to organic chemistry* (3rd ed.). United States of America: John Wiley & Sons. Inc.

Casey, A., Constantin-Teodosiu, D., Howell, S., Hultman, E., & Greenhaff, P. L. (1996). Creatine ingestion favorably affects performance and muscle metabolism during maximal exercise in humans. *American Journal of Physiology: Endocrinology and Metabolism, 271*(1), E31-E37.

Chang, T. W., & Goldberg, A. L. (1978). The metabolic fates of amino acids and the formation of glutamine in skeletal muscle. *The Journal of Biological Chemistry, 253*(10), 3685-3695.

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology, 18*, 117-143.

Clarke, K. R., & Green, R. H. (1988). Statistical design and analysis for a 'biological effects' study. *Marine Ecology- Progress Series, 46*(213), 226.

Clarke, K. R., & Warwick, R. M. (2001). *Change in marine communities: An approach to statistical analysis and interpretation* (2nd ed.). Plymouth Marine Laboratory, Plymouth, U.K.: Primer -E Ltd.

Cyr, N. E., & Romero, L. M. (2007). Chronic stress in free-living european starlings reduces corticosterone concentrations and reproductive success. *General and Comparative Endocrinology, 151*, 82-89.

Dadmarz, M., Burg, C. V. D., Milakofsky, L., Hoffort, J. M., & Vogel, W. H. (1998). Effects of stress on amino acids and related compounds in various tissues of fasted rats. *Life Sciences, 63*(16), 1485-1491.

Feldberg, W., & Vogt, M. (1948). Acetylcholine synthesis in different regions of the central nervous system. *Journal of Physiology, 107*, 372-381.

Fiehn, O. (2002). Metabolomics- the link between genotypes and phenotypes. *Plant Molecular Biology, 48*, 155-171.

Harper, A. E., Miller, R. H., & Block, K. P. (1984). Branched chain amino acid metabolism. *Annual Review of Nutrition,* (4), 409-454.

Harris, H. J. J. (1970). Evidence of stress response in breeding blue-winged teal. *The Journal of Wildlife Management, 34*(4), 747-755.

Hwang, T., & Shaka, A. J. (1995). Water suppression that works. excitation sculpting using arbitrary waveforms and pulsed field gradients. *Journal of Magnetic Resonance, 112*, 275-279.

Johnson, D. H., & Grier, J. W. (1988). *Determinants of breeding distribution of ducks*. University of Nebraska- Lincoln: USGS Northern Prairie Wildlife Research Center.

Landaas, S., & Petersen, J. E. (1975). Clinical conditions associated with urinary excretion of 2-hydroxybutyric acid. *Scandinavian Journal of Clinical and Laboratory Investigation, 35*(3), 259-266.

Le Maho, Y. L., Karmann, H., Briot, D., Handrich, Y., Robin, J. P., Mioskowski, L., et al. (1992). Stress in birds due to routine handling and a technique to avoid it. *American Journal of Physiology Regulatory Integrative and Comparative Physiology, 263*, R775-R781.

Milakofsky, L., Hare, T. A., Miller, J. M., & Vogel, W. H. (1985). Rat plasma levels of amino acids and related compounds during stress. *Life Sciences, 36*, 753-761.

Miller, M. G. (2007). Environmental metabolomics: A SWOT analysis (strengths, weaknesses, opportunities, and threats). *Journal of Proteome Research, 6*, 540-545.

Ni, Y., Su, M., Lin, J., Wang, X., Qiu, Y., Zhao, A., et al. (2008). Metabolic profiling reveals disorder of amino acid metabolism in four brain regions from a rat model of chronic unpredictable mild stress. *FEBS Letters, 582*, 2627-2636.

Nijdam, E., Delezie, E., Lambooij, E., Nabuurs, M. J. A., Decuypere, E., & Stegman, J. A. (2005). Comparison of bruises and mortality, stress parameters, and meat quality in manually and mechanically caught broilers. *Poultry Science, 84*, 467-474.

Premkumar, R. (2012). *Comparative evaluation of 1H NMR spectroscopy as a novel tool for assessing stress with traditional methods of blood corticosterone levels and heterophil lymphocyte ratio in broiler chickens (Gallus domesticus).* Unpublished MS Thesis, University of Saskatchewan, Saskatoon.

Ramsden, J. J. (2009). *Computational biology bioinformatics: An introduction* (2nd ed.). London: Springer-Verlag London Limited.

Rich, E. L., & Romero, L. M. (2004). Exposure to chronic stress downregulates corticosterone responses to acute stressors. *American Journal of Physiology Regulatory Integrative and Comparative Physiology, 288*, R1628-R1636.

Rochfort, S. (2005). Metabolomics reviewed: A new "omics" platform technology for systems biology and implications for natural products research. *Journal of Natural Products, 68*, 1813-1820.

Rozen, S., Cudkowicz, M. E., Bogdanov, N., Matson, W. R., Kristal, B. S., Beecher, C., et al. (2005). Metabolomic analysis and signatures in motor neuron disease. *Metabolomics, 1*(2), 101-108.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews, 21*(1), 55-89.

Siegel, H. S. (1980). Physiological stress in birds. *BioScience, 30*(8), 529-534.

Stevens, L. (1996). *Avian biochemistry and molecular biology*. New York, USA: Cambridge University Press.

Teague, C. R., Dhabhar, F. S., Barton, R. H., Beckwith-Hall, B., Powell, J., Cobain, M., et al. (2007). Metabonomic studies on the physiological effects of acute and chronic psychological stress in sprague-dawley rats. *Journal of Proteome Research, 6*, 2080-2093.

Viant, M. R., Rosenblum, E. S., & Tjeerdema, R. S. (2003). NMR-based metabolomics: A powerful approach for characterizing the effects of environmental stressors on organism health. *Environmental Science Technology, 37*, 4982-4989.

Viant, M. R. (2003). Improved methods for the acquisition and interpretation of NMR metabolomic data. *Biochemical and Biophysical Communications, 310*, 943-948.

Walker, B. G., Dee Boersma, P., & Wingfield, J. C. (2006). Habituation of adult magellanic penguins to human visitation as expressed through behavior and corticosterone secretion. *Conservation Biology, 20*(1), 146-154.

Walker, B. L., Naugle, D. E., & Doherty, K. E. (2007). Greater sage-grouse population response to energy development and habitat loss. *The Journal of Wildlife Management, 71(8),* 2644-2654.

Zimpfer N.L., Rhodes, W. E., Silverman, E. D., Zimmerman, G. S., & Koneff, M. D. (2009). *Trends in duck breeding populations, 1955-2009*U.S Fish and Wildlife Service.